Evaluation of *in situ* remediation methods in soils contaminated with organic pollutants

Suvi Simpanen

Department of Environmental Sciences
Faculty of Biological and Environmental Sciences
University of Helsinki, Lahti
Finland

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Supervisor
Professor Martin Romantschuk
Department of Environmental Sciences
University of Helsinki
Lahti, Finland

Reviewers
Professor Marja Tiirola
Department of Biological and Environmental Science
University of Jyväskylä
Jyväskylä, Finland

Docent Petri Penttinen
Department of Environmental Sciences
University of Helsinki
Helsinki, Finland

Opponent
Professor Marko Virta
Department of Food and Environmental Sciences
University of Helsinki
Helsinki, Finland

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ABSTRACT

Soil contamination is a result of human activities that allow hazardous substances to accumulate in soil and thereby increase the risk to the environment or to human health. There is an estimate of over 2.5 million contaminated sites in Europe and nearly 24 000 of these are in Finland. The most common soil contaminants are oil hydrocarbons and metals. The main anthropogenic activities that contribute to soil contamination include fuel distribution and storage, industrial activity, waste treatment, shooting ranges and sawmill and impregnation areas. Soil restoration has been done mainly by excavating and treating or placing the contaminated soil elsewhere (*ex situ* -treatment). An alternative method is to treat soil in the contaminated area, *in situ*, without excavation, which reduce both environmental effects and remediation costs. The low use of *in situ* treatments can be explained by a lack of knowledge, lack of experiences, the uncertainty about treatment effectiveness, uncertainty about cleaning performance and the longer time usually needed for *in situ* -remediation.

Various *in situ* remediation methods were evaluated for this PhD project by using laboratory-, pilot- and field scale experiments, and comparing their usefulness and effectiveness in treating soils contaminated with organic contaminants. The study focused on organic contaminants, found most often in contaminated areas, which include: oil hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and chlorinated aromatic hydrocarbons (chlorophenols). The soils used in the study were mainly obtained from a former gas station and wood impregnation areas. In addition, a real fuel spill accident was simulated on a pilot scale, after which the effect of different *in situ* remediation methods were compared. The remediation methods studied in this thesis used the five following approaches: 1) enhancement of microbiological degradation capacity via forest soil addition, 2) biostimulation, i.e. increase of microbiological degradation by improving soil nutrient and oxygen content, 3) enhanced bioavailability of water insoluble contaminants by using a surfactant (methyl-β-cyclodextrin), 4) chemical oxidation with hydrogen peroxide and 5) the use of electrokinetic remediation.

1) The addition of organic forest soil on top of chlorophenol contaminated mineral soil significantly increased chlorophenol degradation under optimal conditions. The positive effect was also seen under field conditions. 2) The most important factor for increasing the degradation of contaminants by microbial action was an improvement of soil nutrient ratios via nitrogen amendment. Biostimulation of freshly oil-contaminated soil caused changes in soil microbial community and decreased the oil leakage through the soil, and it was deemed to be a suitable method to use in groundwater areas. 3) Biostimulation in PAH-contaminated soil reduced the amount of low molecular weight PAH-compounds. Surfactant was useful in the solubilisation and enhanced biodegradation of 4-5 ringed PAH-contaminants. 4) Chemical oxidation was not effective for fresh, oil-contaminated soil but it increased the oil migration through the soil. The method also requires large amounts of reagents and an effective infiltration system. 5) An electric field that was introduced into the dense and wet soil, caused soil dewatering, after which the soil vapor extraction was effective for the removal of volatile organic contaminants.

Biostimulation is applicable to use as a first remediation method to decrease easily degradable, organic contaminants from the soil. The combination of different methods and their sequential use can decrease the need for excavation. Decisions about remediation should be done early enough to make the use of *in situ* methods feasible.

TIIVISTELMÄ

Maaperä pilaantuu kun ihmisen toiminnan seurauksena maaperään päätyy haitallisia aineita, jotka aiheuttavat vaaraa ympäristölle tai ihmisen terveydelle. Pilaantuneiksi luokiteltuja alueita on Euroopassa arvioitu olevan yli 2.5 miljoonaa ja Suomessa lähes 24 000. Yleisimmät pilaantumista aiheuttavat haitta-aineet ovat öljyhiilivedyt ja metallit ja merkittävimpiä pilaantumista aiheuttavia toimintoja polttoaineen jakelu ja varastointi, teollinen toiminta, jätteen käsittely, ampumaradat sekä sahat ja kyllästämöt. Pilaantuneen maaperän kunnostamista on tehty pääosin kaivamalla maamassat ja käsittelemällä tai sijoittamalla ne muualla (*ex situ* –kunnostus). Vaihtoehtoisesti maaperä voidaan kunnostaa *in situ*, paikan päällä ilman kaivamista, mikä vähentää sekä ympäristövaikutuksia että kunnostuskustannuksia. *In situ* –kunnostuksen vähäiseen käyttöön vaikuttavia tekijöitä ovat muun muassa tiedon ja kokemusten puute, epävarmuus tekniikoiden toimivuudesta ja puhdistustuloksesta sekä kunnostuksen pidempi kesto.

Tässä väitöskirjatyössä tutkittiin laboratorio-, pilot- ja kenttämittakaavan kokeilla erilaisia *in situ* soveltuvia kunnostusmenetelmiä ja arvioitiin niiden toimivuutta ja tehoa orgaanisilla saasteyhdisteille pilaantuneiden maiden puhdistumisessa. Tutkimuksessa keskityttiin yleisimmin maaperistä löytyviin orgaanisiin saasteyhdisteisiin öljyhiilivetyihin, polysyklisiin aromaattisiin hiilivetyihin (PAH) sekä kloorattuihin aromaattisiin hiilivetyihin (kloorifenolit). Tutkimuksessa käytetyt maa-ainekset olivat peräisin pääosin vanhoilta huolto-asema- ja puun kyllästämö alueilta. Lisäksi pilot mittakaavan kokeessa mallinnettiin oikeaa polttoaineonnettomuutta ja vertailtiin eri menetelmien tehokkuutta tuoreen saastemaan puhdistamisessa. Tutkittavat kunnostusmenetelmät perustuivat 1) mikrobiologisen hajotuskapasiteetin parantamiseen humuslisäyksen avulla, 2) biostimulaation eli mikrobiologisen hajotustoiminnan lisäämiseen maan ravinne- ja happipitoisuutta parantamalla, 3) veteen liukenemattomien yhdisteiden biosaatavuuden parantamiseen pinta-aktiivisen aineen (metyyli-β-syklodekstriini) avulla, 4) kemialliseen hapetukseen vetyperoksidin avulla sekä 5) elektrokinetiikan käyttöön kunnostustoimien tehostamiseksi.

1) Orgaanisen metsämaakerroksen lisäys kloorifenoleilla saastuneen mineraalimaan päälle lisäsi kloorifenolien hajoamista optimiolosuhteissa merkittävästi. Myös kenttäolosuhteissa humuksen positiivinen vaikutus oli havaittavissa. 2) Tärkein mikrobihajotusta edistävä tekijä oli saastuneen maan ravinnetasapainon parantaminen typpilisäyksellä. Tuoreessa öljyllä saastuneessa maassa biostimulaatio muutti maan mikrobiyhteisön koostumusta sekä vähensi maan läpi kulkeutuvan öljyn määrää, osoittautuen näin soveltuvaksi menetelmäksi myös pohjavesialueille. 3) PAH-maassa biostimulaation avulla saatiin pienimolekyyliset PAH-yhdisteet vähenemään. Pinta-aktiivinen aine osoittautui kannattavaksi 4-5 renkaisten PAH-yhdisteiden irrottamisessa ja hajottamisessa maasta. 4) Kemiallinen hapetus ei saanut aikaan tuoreen öljymaan puhdistumista mutta lisäsi öljyn kulkeutumista maan läpi. Menetelmä vaatii suuria reagenssiannoksia ja tehokasta reagenssin syöttötapaa. 5) Maahan muodostetun sähkökentän avulla saatiin tiivis savimaa kuivumaan, jolloin huokoskaasukäsittely haihtuvien orgaanisten yhdisteiden poistamiseksi tehostui.

Biostimulaatio sopii ensisijaiseksi keinoksi vähentää orgaanisia helposti hajoavia saasteyhdisteitä maasta. Eri menetelmien yhdistelmällä ja vuorottaisella käytöllä voidaan vähentää tai poistaa massanvaihdon tarvetta. Päätökset pilaantuneiden alueiden kunnostuksesta tulisi tehdä aikatauluilla, jotka sallivat *in situ* –menetelmien käytön.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Aki Sinkkonen, Sari Kauppi, Suvi Simpanen, Anna-Lea Rantalainen, Rauni Strömmer, Martin Romantschuk. 2013. Layer of organic pine forest soil on top of chlorophenol-contaminated mineral soil enhances contaminant degradation. Environmental Science and Pollution Research 20:1737-1745.

II. Suvi Simpanen, Riikka Mäkelä, Juha Mikola, Hannu Silvennoinen, Martin Romantschuk. 2016. Bioremediation of creosote contaminated soil in both laboratory and field scale: Investigating the ability of methyl-β-cyclodextrin to enhance biostimulation. International Biodeterioration & Biodegradation 106:117-126

III. Suvi Simpanen, Mari Dahl, Magdalena Gerlach, Anu Mikkonen, Vuokko Malk, Juha Mikola, Martin Romantschuk. Biostimulation proved to be the most efficient method in the comparison of *in situ* soil remediation treatments after a simulated oil spill accident. 2016. Environmental Science and Pollution Research DOI 10.1007/s11356-016-7606-0

IV. Suvi Simpanen, Dan Yu, Riikka Mäkelä, Harri Talvenmäki, Aki Sinkkonen, Hannu Silvennoinen, Martin Romantschuk. Soil vapor extraction of wet gasoline contaminated soil made possible by electro-osmotic dewatering – lab simulations applied at a field site. Submitted manuscript.

THE AUTHOR'S CONTRIBUTION

I. SS was involved in the planning and conducting the field experiment. SS analyzed and interpreted the corresponding results and participated in the writing of the paper.

II. SS participated in the planning and setting-up the experiments, and was responsible for analyzing and interpreting the results. SS wrote the paper and is the corresponding author. The co-authors revised the paper.

III. SS planned and conducted the experiment, was responsible for analyzing and interpreting the results, wrote the paper and is the corresponding author. The co-authors revised the paper.

IV. SS planned and set up the laboratory experiment and participated in the implementation of the field experiment. SS was responsible for analyzing and interpreting the results and wrote the paper together with MR.

ABBREVIATIONS USED IN THE THESIS:

AC	Alternating current
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BTEX	Benzene, toluene, ethylbenzene, and xylene
CaO_2	Calcium peroxide
CD	cyclodextrin
C:N	Carbon:Nitrogen ratio
C:N:P	Carbon:Nitrogen:Phoshorus ratio
DC	Direct current
EPA	U.S. Environmental Protection Agency
ETBE	Ethyl tertiary butyl ether
H_2O_2	Hydrogen peroxide
ISCO	*In situ* chemical oxidation
ISCR	*In situ* chemical reduction
LH-PCR	Length heterogeneity PCR
MANOVA	Multivariate analysis of variance
MgO_2	Magnesium peroxide
MTBE	Methyl tertiary butyl ether
NAPL	Non-aqueous phase liquid
O_3	Ozone
PAH	Polycyclic aromatic hydrocarbons
PCP	Polychlorophenol
PeCP	Pentachlorophenol
POP	Persistant organic pollutant
SVE	Soil vapor extraction
TAEE	Tertiary amyl ethyl ether
TAME	Tertiary amyl methyl ether
TCA	Trichloroanisole
TCP	Trichlorophenol
TeCP	Tetrachlorophenol
VOC	Volatile organic compound

1. INTRODUCTION

1.1 Soil contamination

Soil is the top layer of the land on earth´s surface and it is composed of weathered rock particles i.e. minerals, organic matter, water, air and living organisms. It can be considered to be a non-renewable resource due to the extreme slowness of the soil-forming processes (SOER 2010, JRC 2012). Soil has an essential role in many life maintaining functions including food production, non-food biomass production, nutrient cycling, and purifying water supplies (SOER 2010, JRC 2012). Various human activities have caused soil degradation, which has become an increasing, widespread problem and contamination is one of the main threats to soil. Large scale soil contamination can be said to have started in the 19th century due to the wide-scale industrialization and rapid technological developments (Swartjes 2011). The growing awareness of the problems related to soil contamination has only occurred in the last few decades.

A contaminated site is an area, where hazardous substances caused by human activities are present in quantities that endanger or pose a risk to human health or the environment (Ministry of Environment 2014, SOER 2010). Local soil contaminations vary in size and intensity, and have a heterogeneous contamination patterns. These result from intensive industrial activities, inadequate waste disposal, mining, military activities or accidents (SOER 2010, Swartjes 2011). In diffuse soil contamination cases, the contaminant source can be dispersed over a large area via atmospheric deposition, flood events or by agricultural activities (SOER 2010, Swartjes 2011). Two significant reasons for diffuse contamination is the over-application of pesticides and of mineral fertilisers (JRC 2012).

The numbers and variety of different soil contaminants are vast as are the types of contamination sources. The main categorization can be made between organic and inorganic contaminants and further sub-groupings of these contaminants are based on their chemical structures (Table 1). The chemical structure also affects the compound´s behavior in the environment; e.g. how persistent, volatile or soluble it is. The most hazardous contaminants are the persistent organic pollutants (POPs), which are resistant to chemical, biological and photolytic degradation.

Evaluation of the extent of soil contamination is restricted by the variable inventory and mapping systems used by different countries. The number of estimated potentially contaminated sites in Europe is over 2.5 million of which about 14% are actually identified (Liederkerke et al. 2014, Panagos et al. 2013). In the United States the National Priorities List of the U. S. Environmental Protection Agency (EPA) contains 1383 proposed or identified most serious uncontrolled or abandoned hazardous waste sites (EPA 2016). A systematic inventory of contaminated sites was implemented in Finland in the 1980s. Nowadays, the information on contaminated, potentially contaminated and cleaned up land areas in Finland are collated in the MATTI soil condition database, which was created and is run by the Finnish environmental administration, and shows that the number of recognized sites is nearly 24 000 (Pyy et al. 2013). The main contaminant categories that contribute to the local soil contamination in Europe and in Finland are heavy metals and mineral oil (Liederkerke et al. 2014, Pyy et al. 2013). The main activities that contribute to the soil contamination in Europe are waste

disposal and treatment and industrial and commercial activities (Fig. 1). In industrial sector the metal industry and in service sector the gasoline stations are the main sources (Panagos et al. 2013).

Table 1. Main soil contaminant categories including examples of soil contaminant compounds and contamination sources (Valentin et al. 2013, Swartjes 2011, Reinikainen 2007)

Contaminants	Examples	Source of contamination
Inorganic		
Metals/metalloids	Pb, Cd, Hg, Zn, Cr, Cu, As	gasoline stations, shooting ranges (Pb), mining and foundry activities (As, Cd, Pb, Zn), agricultural practices (Cd, As), batteries (Pb, Cd), wood preservation (Cr, Cu, As), fungicides (Hg), metal industry (Cr, As, Hg, Cd), waste combustion and treatment (As, Hg)
Non-metals	cyanide nutrients (nitrate, phosphate, potassium salts, sulfate)	gas work sites, electroplating processes agricultural practices
Organic		
Chlorinated		
Aromatics	chlorophenols (e.g. TCP, TeCP, PCP)	wood preservatives, pesticides, disinfectants
	polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF)	incineration of chlorine-containing waste, impurities in wood preservatives or PCB products
	polychlorinated biphenyls (PCB)	hydraulic oils and lubricants, electrical insulators and heat transfer agents, paints
	dichlorodiphenyltrichloroethane (DDT)	insecticides
Aliphatics	dichloromethane (DCM)	cleaning agent in plastic industry, solvent in paints, glues and strippers
	dichloroethylene (DCE), trichloroethylene (TCE), tetrachloroethylene (PCE)	dry-cleaning industry, solvent agent in industry
	vinyl chloride	PVC-production, degradation product of chlorinated hydrocarbons TCE and PCE
Non-chlorinated		
Oil hydrocarbons	alkanes, alkenes, cycloalkanes	oil industry, gasoline stations
Polycyclic aromatic hydrocarbons (PAHs)	naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)-fluoranthene, benzo(k)- fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, ideno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene	oil industry, incomplete combustion of carbon-containing fuels, wood preservation
Monomeric aromatic hydrocarbons	benzene, toluene, ethylbenzene, xylenes (BTEX)	oil industry, gasoline stations, solvents
Nitroaromatics	2,4,6- trinitrotoluene (TNT), nitrobenzene, nitrophenols, atrazine	explosive industry, military use, pesticides, herbicides

IN EUROPE IN FINLAND

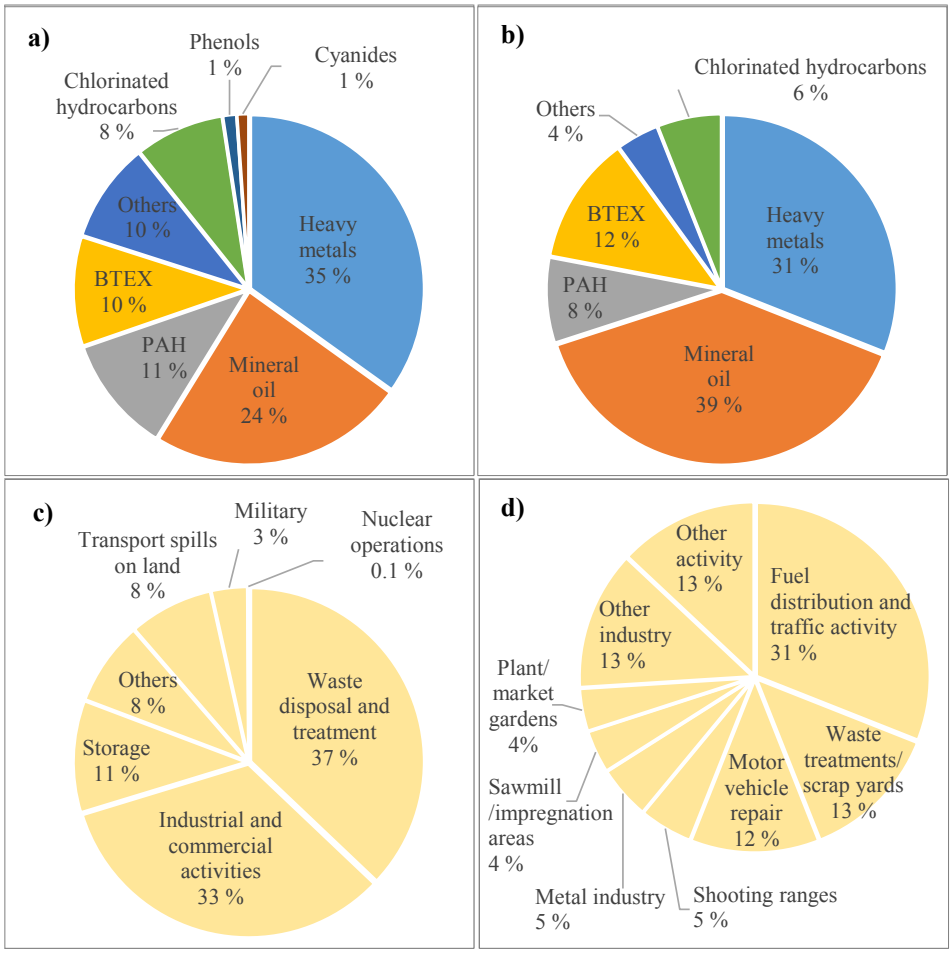

Figure 1. Distribution of contaminants and activities that contribute to soil contamination in Europe (a,c) and in Finland (b,d). Redrawn and modified from Liederkerke et al. 2014 (a-c) and FEA 2014 (d).

1.1.1 Fuel contaminants

Nowadays crude oil or petroleum products are the most used chemicals that cause environmental and health related concerns. Contamination of the environment with these products has been vast. Reasons for soil contamination by fuels are usually leakages that occur during storage or transportation accidents.

Conventional fuels (diesel and gasoline) are produced in the distillation of crude oil and both fuels consist of a heterogeneous mixture of hydrocarbons and other compounds depending on the production process and origin of crude oil (Speight and Arjoon 2012). Diesel consists a mixture of C_8-C_{40} hydrocarbons, which predominantly are C_{10}-C_{22} aliphatic (paraffins, naphthenes, olefins) and aromatic hydrocarbons (Speight and Arjoon 2012, Pitz and Mueller 2011,

Bacha et al. 2007). Small amounts of heteroatoms i.e. nitrogen-, sulfur-, oxygen and metals containing compounds and different kinds of additives are also present (Bacha et al. 2007).

Gasoline is composed of C_4-C_{12} hydrocarbons of which 41-62% are aliphatics (straight-chained and branched alkanes, alkenes, cycloalkanes) and rest are aromatics especially benzene, toluene, ethylbenzene, and xylene (BTEX) compounds (Speight and Arjoon 2012, Gibbs 2009, Chen 2007). Ethers such as methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE) and tertiary amyl methyl ether (TAME) or alcohols (ethanol and methanol) have been used as oxygenates to enhance the octane number of gasoline (Gibbs 2009).

1.1.2 Wood preservatives

Wood-preserving and impregnation processes have caused soil contamination in numerous sawmill and other industrial areas. Preservative components can be released into the environment also from treated wood products. The most used chemicals have been creosote, pentachlorophenol and chromated copper arsenate (CCA) (Mueller 1989).

Creosote is a fungicide, insecticide, and sporicide that is mainly used in the pressure treatment of railroad ties and utility poles (EPA 2008). It is a complex mixture of hundreds of chemicals of which 200-250 are identifiable substances, and it is formed by the fractional distillation of crude coal tars in the range of 200-400 °C (EPA 2008, Melber 2004). The creosote component types and concentrations vary depending on the origin of the coal and the nature of the distilling processes. The main component classes are PAHs (85-90%) phenolic compounds (2-17%) and N-, S- and O-containing heterocycles (1-8%) (Melber 2004, Mueller 1989). Nowadays, the use of creosote and creosote treated wood has been restricted in Europe (REACH regulations EC No. 1907/2006 and No. 552/2009).

Chlorophenols have been used in wood preservation as fungicides to prevent wood staining. In Finland a chlorophenolic compound, trade named Ky-5, was used on a large scale in saw-mill areas during 1940-1980s until it was banned on 1988 (Lyytikäinen 2004). It consisted of powdery sodium salts of 2,3,4,6-tetrachlorophenol (70-80%), 2,4,6-trichlorophenol (5-15%) and pentachlorophenol (5-15%) and it was used as a 1-5% aqueous solution (Reinikainen 2007, Lyytikäinen 2004). The Ky-5 preservative also contained small amounts of dibenzo-p-dioxins, dibenzofurans, diphenyl ethers and phenoxyphenols, which formed as impurities in the production process.

1.2 Soil remediation techniques

Soil remediation can be performed *in situ*, in its original place, without soil excavation or *ex situ*, excavating a contaminated soil and treating it on the same site (*ex situ,* on site) or transporting it for cleaning or to landfills (*ex situ,* off-site) (EUGRIS 2007, EPA 2007). Treatment mechanisms can be based on biological, physical, chemical, electrical or thermal contaminant removal (Table 2) of which biological technologies are estimated to be the most cost efficient (Juwarkar et al. 2010). *In situ* remediation is applied in a natural, heterogeneous and less controlled environment. Due to this they typically require longer treatment times, the uniformity of the treatment effectiveness in soil might be uncertain and the monitoring of the remediation progress might be difficult (EUGRIS 2007). However, *in situ* treatments are most often the more cost effective options, they avoid the contaminant spreading due to excavation and transport, they require less technological equipment and are

relatively simple to apply in comparison *ex situ* methods (Tomei 2013). Despite the fact that *ex situ* remediation is fast, predictable and efficient way for soil decontamination, the problem is actually transferred from one site to another. Excavation and off-site treatment consume nonrenewable natural resources and energy due to the use of heavy machinery, transport over the long distances and replacement of the removed soil with a clean soil (Ministry of Environment 2014). In addition, *ex situ* remediation causes emissions and changes in the landscape and ecosystem. Soil remediation using completely or partly *in situ* methods would reduce hazardous environmental effects.

The development of *in situ* remediation started in the early 1990s when many sites seemed to be too large for excavation and subsequent treatment (Grotenhuis and Rijnaarts 2011). More recently, the use of *in situ* applications have increased especially in the U.S., where *in situ* treatment was chosen for half of the Superfund cases during the latest documented period in 2009-2011. The most frequent techniques were soil vapor extraction, chemical treatment and solidification/stabilization (EPA 2013). However, in Europe *in situ* remediation is still relatively low; in most countries *in situ* based techniques were used for less than 30% of reported contaminated sites in 2011 (Fig. 3) (Liederkerke et al. 2014). The low use of *in situ* methods is often explained by doubts regarding their efficiency and risk reduction, long-term ecological effects, time consumption, and lack of data on their suitability especially in Nordic conditions (Sorvari et al. 2009).

1.2.1 Biological methods

The simplest approach for contaminated site management is natural attenuation, which actually starts to take place in the moment the contaminants come into contact with the soil. It includes destructive processes such as contaminant degradation by soil indigenous microorganisms (i.e. biodegradation/bio-remediation) and non-destructive processes like dispersion, dilution, volatilization, sorption and immobi-lization of contaminants (Peter et al. 2011). Biodegradation can be stimulated or accelerated by improving the environmental conditions in which case the soil management is known as "enhanced natural attenuation". In biostimulation nutrients or other substances are added into the soil to increase the activity and growth of the indigenous microbial population, whereas in bioaugmentation pre-grown microbial cultures (single strain or consortia) are added into the soil (Vogt and Richnow 2014, Peter et al. 2011, Speight and Arjoon 2012). Although bioaugmentation using enriched micro-organisms from contaminated sites has been proven to be an effective approach for remediation of oil-contaminated soil (Alisi et al. 2009, Bento et al. 2005), contradictory results on its effectiveness have also been published (Kauppi et al. 2011, Thomassin-Lacroix et al. 2002). The reasons for the lack of effectiveness of bioaugmentation are a failure of inoculated microorganisms to adapt to changes in environmental conditions of the new site, competition with autochthonous microbes and predation by protozoa (Tyagi et al. 2011, Gentry et al. 2004a). The problems related to bioaugmentation with cultured microbes can be avoided by using a soil inoculant containing the indigenous degrader population, which can be reintroduced into the contaminated site (Gentry et al. 2004b). The degradation capacity of the soil inoculant can evolve naturally (organic forest soils) or via previous exposure to contaminant (Kauppi et al. 2012, Gentry et al. 2004 b).

Soil microbial degradation capacity is a prerequisite for efficient bioremediation.

Other factors that affect the soil remediation processes and biodegradation rate are temperature, moisture, pH-value, soil type, oxygen or other electron acceptor (sulfate, carbonate, iron, nitrate) content, nutrient content, chemical structure, bioavailability and the quantity of contaminants and toxic or interfering metabolite content (Vogt and Richnow 2014, Grotenhuis and Rijnaarts 2011, Margesin 2000, Romantschuk et al. 2000).

Aerobic biodegradation is more efficient than anaerobic biodegradation (Schreiber et al. 2004), and various methods to increase oxygen level in soil are used. In bioventing a low air flow is injected into the soil to maximize the biodegradation process. Other air extracting/injecting methods whereby the main purpose is to volatilize contaminants using a higher air flow, can also stimulate biodegradation (Nathanail et al. 2007). Soil porosity can be increased by mixing bulking agents such as wood chips, straw or sawdust in with the contaminated soil and this has been found to be a sufficiently effective aeration method in oil-contaminated soil piles (Kauppi et al. 2011, Jørgensen 2000). Oxidants such as hydrogen peroxide (H_2O_2) and ozone (O_3), used in chemical oxidation processes, produce oxygen when they decompose, which can also benefit the aerobic biodegradation of contaminants (Sutton 2011, ITRC 2005). Problems related to H_2O_2 use in soil aeration are the rapid depletion of H_2O_2 after contact with humic substances or metals and toxicity for microbes at high concentrations (Cassidy and Irvine 1999). A slower release of oxygen is achieved with solid oxygen sources, such as calcium peroxide (CaO_2) and magnesium peroxide (MgO_2), that decompose and release oxygen in the presence of water (Cassidy and Irvine 1999). Amendment with CaO_2 was found to be an effective method for the elevation of oxygen levels in sediments, which enhanced organic matter degradation by microbes (Nykänen et al. 2012). Under anaerobic conditions, diesel degradation was observed to be the most efficient when various electron acceptors (sulfate, nitrate, carbonate) were used simultaneously whereas individually used, sulfate was the best of these three choices (Boopathy 2004).

When soil is contaminated with organic contaminants, the soil carbon:nitrogen ratio (C:N) is in an imbalance due to the excess of carbon. Various suggestions for optimal C:N ratio have been given that range from values 5.5:1 to 560:1 (Shewfelt et al. 2005). Generally the carbon:nitrogen:phosphorus (C:N:P) ratio 100:10:1 has been recommended as the optimal ratio for biostimulation but this should only be considered as a suggestive guide value (Álvaretz 2015, Chaîneua 2005). The optimal nutrient level depends on the type of nutrient product, microbial assimilation efficiency, bioavailability of contaminant and characteristics of the contaminated environment (Walecka-Hutchison et al. 2006). Nitrogen derived from ammonium (NH_4-N) has been considered in many studies to be a more effective nitrogen source compared to nitrogen derived from nitrate (NO_3-N) due to its short lag time before microbial degradation and enhanced degradation rate (Shewfelt et al. 2005). However, the use of ammonium usually requires an addition of a buffer if the nitrification rate in the soil is high, because the rapid transformation of ammonia to nitrate leads to soil acidification and hence an inhibition of degradation (Peltola et al. 2006, Shewfelt et al. 2005). Also, fertilization dosage is an important factor when using ammonium and N-sources releasing ammonium easily. A high urea load in an oil-waste landfarming field was found to increase soil ammonium concentration and pH, which inhibited the nitrification what for the slow-releasing nitrogen source, methylene urea, was more suitable for bioremediation (Peltola et al. 2006). Methylene urea also induced less

nitrification than urea, which decreased the risk of the nitrate leaching into the groundwater (Peltola et al. 2006). Adverse effects of excessive nitrogen input in diesel contaminated soil were also reported by Walecka-Hutchison et al. (2006) who proposed that fertilization should not be based solely on soil C:N ratios, but due to the variable soil textures it should also include soil pore water nitrogen (mg N/ kg soil H_2O), which takes into account the soil moisture content. Excessive nitrogen input, especially on coarse-texture soils with low water-holding capacities, increase fertilizer solutes and thus salinity of the pore water. This increase in salinity causes a depression in soil water potential, which expose bacteria to osmotic stress and reduce microbial degradation (Walecka-Hutchison et al. 2007 and 2006).

Biodegradation can weaken due to the low bioavailability of contaminant despite there being an optimal nutrient content in the soil. Compounds (e.g. PAHs and chlorinated hydrocarbons) with low water solubility, complex molecular structure and high molecular weight adsorb efficiently onto soil particles and make them recalcitrant and difficult to degrade (Mudhoo and Mohee 2012, Gao et al. 2009). Hydrophobic forces on the soil particle surfaces induce the physical adsorption of organic contaminant into soil and it can exist in the soil as a particulate pollutant, liquid film, adsorbed onto or into soil or within the soil macro or micro pores (Paria 2008). Soil properties such as organic matter content, cation exchange capacity, micropore volume, soil texture and surface area have impacts on soil-contaminant interactions (Chung and Alexander 2002). The sorption of the contaminant onto soil is enhanced by a long contact time, and this decreases bioavailability of contaminant (Haritash and Kaushik 2009, Hatzinger and Alexander 1995). An increase in contaminant bioavailability has been achieved by thermal treatments (Bonten et al. 1999), ozonation (Haapea and Tuhkanen 2006) and Fenton-like chemical oxidation (Kulik et al. 2006). Also organic solvents (Bonten et al. 1999), vegetable oils (Mao et al. 2013, Gong et al. 2005) and synthetic or biosurfactants (Rodríquez-Escales et al. 2013, Pei et al. 2010) have been tested for the desorption of hydrophobic pollutants from soil and the increase of biodegradation. Surface-active compounds i.e. surfactants are amphiphilic molecules that contain hydrophobic and hydrophilic portions. The properties of the latter determine whether the surfactant is anionic, cationic, zwitterionic or non-ionic (Mulligan 2009, Paria 2008). The enhanced biodegradation by surfactants is caused by an increase in the contaminant dissolution and an increase in microbial cell surface hydrophobicity, which change the affinity between the microbial cell and the contaminant (Paria 2008). Surfactants can also improve the biodegradation by reducing toxicity of the co-contaminants. Chrzanowski et al. (2011) found that interactions between surfactant and chlorophenols reduced their toxicity improving the biodegradation of diesel hydrocarbons in effluent containing petroleum and chlorinated phenols. Biosurfactants are surface-active compounds of biological origin. They can be glycolipids, lipopeptides, lipoproteins, phospholipids or fatty acids that are produced by micro-organisms. The most studied biosurfactant type to date is the rhamnolipids of the glycolipid group (Ławniczak et al. 2013, Chrzanowski et al. 2012, Mulligan 2009). Cyclodextrines (CD) can also be considered as biosurfactants because they are produced by bacteria in the enzymatic degradation of starch (Morillo 2012, Del Valle 2004). They have cylinder-like structure with a hydrophobic interior and a hydrophilic exterior (Singh et al. 2007) and the most used derivative β-CD has a half-life ranging from 0.5-1.5 years which is

suitable for soil remediation purposes (Gruiz et al. 2010). Advantages of biosurfactants are their biodegradability and their relative non-toxicity. These properties can also be disadvantageous as they can facilitate micro-organisms using the biosurfactant as a primary carbon source instead of the targeted contaminant. Rhamnolipids have been found to be biodegraded in preference to diesel oil (Ławniczak et al. 2013) while β-CD as the sole carbon source did not support microbial growth when its efficacy was investigated for microbial hydrocarbon degradation (Bardi 2000).

1.2.2 Chemical methods

In situ chemical oxidation (ISCO) is a method by which rapid reaction with the organic contaminant is achieved by introducing a liquid or gaseous oxidizing agent into the soil (Nathanail et al. 2007, ITRC 2005). As a result, the contaminant degrades completely into carbon dioxide and water or transforms into a more biodegradable form. The main oxidants used in soil remediation are permanganate, persulfate, peroxide and ozone (ITRC 2005). *In situ* chemical reduction (ISCR) involves a reducing agent. For example, zero valent iron is used for contaminant destruction (Dolfing et al. 2008). Hydroxyl radicals ($OH^{-}\cdot$) are very strong oxidizing agents (oxidation potential of 2.8 V) which are formed in the decomposition of hydrogen peroxide (H_2O_2) catalyzed by ferrous iron (Fe^{2+}) at pH 2.5-3.5 (Baciocchi 2013, ITRC 2005). This reaction, called Fenton´s reaction, is utilized in soil remediation commonly as a modified version. In modified Fenton´s process, high concentrations of H_2O_2 or calcium peroxide can be used. Chelating agents for iron solubilization or H_2O_2 stabilization are possible. In addition, reaction can be done in soil natural pH or soil natural iron content can be used as catalyst (Baciocchi 2013, Goi et al. 2009, Goi et al. 2006). As a nonselective process, ISCO alters soil subsurface conditions and impacts soil microbial populations. On contrary, it has also been found to increase contaminant biodegradability, oxygen content and nutrient release in soil. Therefore, a coupled ISCO and bioremediation treatment can be a more effective strategy than either treatment alone (Sutton et al. 2011).

1.2.3 Physical methods and electroremediation

Physical *in situ* remediation methods are based mainly on volatilization or sorption of contaminants (Table 2). Moreover, electricity can be utilized in soil remediation. This involves an electric field in the contaminated area being created by applying low voltage direct current (DC) or alternating current (AC) between electrodes placed into the soil (Moghadam et al. 2016, Gill et al. 2014). An electric field induces electrokinetic transport (including electroosmosis, electro-migration and electrophoresis) and electrochemical reactions in the soil (Ferrarese 2010). Electroosmosis is a movement of liquid or soil pore water usually in the direction of anode to cathode. The electric field causes cations to desorb from negatively charged soil surface and migrate towards the cathode and carry water along with them (Niroumand et al. 2012, Pazos et al. 2010, Nathanail et al. 2007). In electromigration ions and ion complexes move toward opposite charged electrodes whereas electrophoresis is a movement of charged, dissolved or suspended particles in an electric field (Gill et al. 2014, Niroumand et al. 2012). In addition, electrochemical reactions, such as electrolysis of water molecules occurs at the electrodes and generates H^{+} ions and oxygen gas at the anode and OH^{-} ions and hydrogen gas at the cathode (Pazos et al. 2010, Reddy 2010). This causes an acidic environment around the anode and alkaline

environment around the cathode. Changes in soil pH and migration of ionic products (H^+ and OH^-) affect the geochemical and remediation processes (Reddy 2010). Electrokinetic remediation in combination with other treatment technologies can be utilized for the removal of both organic and inorganic contaminants (Gill et al. 2014, Reddy 2010). Enhanced biostimulation (Suni et al. 2007), bacteria mobilization (Mena et al. 2012, Da Rocha et al. 2009, Suni et al. 2004), increased contaminant bioavailability (Niqui-Arroyo and Ortego-Calvo 2010) and oxygen supply to soil (Ramírez et al. 2014) have been achieved with electro-bioremediation treatments.

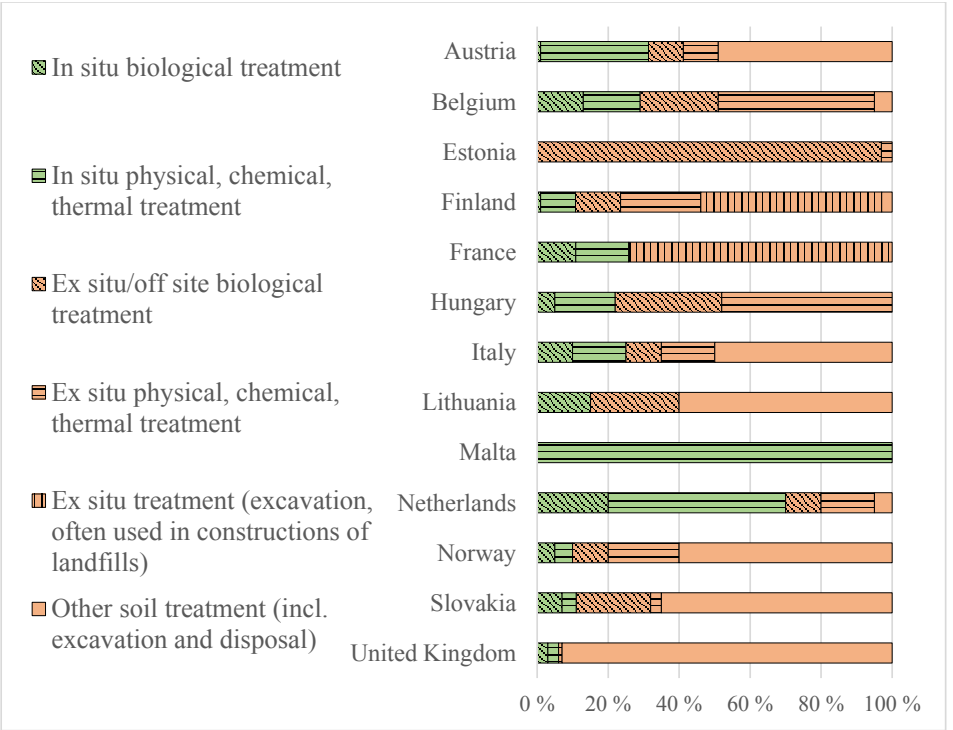

Figure 2. Remediation treatments used for contaminated soil in some European countries reported in the year 2011. Modified from EEA 2014.

Table 2. Description of various *in situ* and *ex situ* soil remediation methods including some of their benefits and disadvantages (EPA 2013, Tomei 2013, Sorvari et al. 2009, Nathanail et al. 2007, EPA 2007, Penttinen 2001).

***In situ* method**		**Principle**	**Benefits**	**Disadvantages**
Biological				
	Monitored natural attenuation	contaminant reduction by natural (biological, physical and chemical) processes, efficacy of soil cleanup and risk reduction are monitored	+ cost-effective	-long-term process -suitable only for some contaminants
	Biostimulation	creating a favorable environment for microbial contaminant degradation, includes the addition of nutrients, oxygen, electron acceptor/donor and controlling the temperature and pH	+ cost-effective	- suitable only for degradable contaminants - low contaminant bioavailability reduces degradation efficiency
	Bioaugmentation	addition of enriched/acclimated microbes having a degradation capacity to specific contaminant		-possibly ineffective due to the failure adaptation of microbes to environmental conditions
	Bioventing, (biosparging)	injection of air/oxygen above (or below) the water table using low pressure and venting rate optimized to maximize biodegradation	+ minimal site disturbance	- unsuitable in low-permeable or heterogeneous soils
	Phytoremediation	use of living plants to remove, degrade, contain or immobilize the contaminants	+low-cost, sustainable method	- long treatment time (years, decades)
Chemical				
	Chemical oxidation/ reduction	injection of oxidizing or reducing agents into soil to cause a complete or partial degradation of contaminant	+ rapid degradation also in high contaminant concentrations	- nonselective degradation can cause negative environmental impacts
Physical				
	Soil vapor extraction	applying a high vacuum to the soil to induce air flow and remove of volatile contaminants	+ cost-effective, well-known technique	-low efficiency in wet and dense soil
	Electro-remediation	inducing an electric field into the soil to cause movement and desorption of charged contaminants	+/- most efficient in fine grained, wet soils + suitable also to inorganic contaminants	
	Encapsulation Solidification Stabilisation	reduction of contaminant mobility and accessibility by coating the soil or via reaction with binding reagent (cement, fly ash, lime, polymers)	+ can be quick and suitable for sources that otherwise untreatable	-does not remove contaminants

Ex situ method	Principle	Benefits	Disadvantages
Excavation and off-site disposal	removal and transportation of soil elsewhere for treatment or landfill use	+ fast and effective	-expensive, cause environmental effects
Composting	building soil piles, which are aerating and fertilizing to enhance biodegradation	+ low costs	-suitable only for some organic contaminants
Bioreactors	treating the soil in enclosed reaction vessels to optimize the biodegradation process and reduce treatment time	+ easy to control due to enclosed system	-not suitable for inorganic contaminants
Land farming	regular cultivation of contaminated soil in a treatment bed, which improves soil structure and oxygen supply	+/- used mostly in oil contaminated soils	- requires large areas and long treatment times, contaminant decrease might be low
Soil washing	separation of contaminants/contaminated soil particles from uncontaminated soil in a water-based system, separation based on different physical properties of contaminated particles or chemical dissolution of contaminants	+ suitable for a range of contaminants	- efficacy dependent on soil type and nature of contaminant -may require additional processes for water treatment
Incineration	destruction of contaminants using high temperatures (800-1300°C) to volatilize and combust hazardous waste	+ removal efficiency 100%	- expensive and high energy use
Thermal desorption	use of heat (90-560°C) to separate contaminant from soil by volatilization, followed by destruction of contaminants from vapors	+ potential for high contaminant removals	- might need pre-treatment of soil (dewatering, screening)

2. AIMS OF THE STUDY

The general objective of this study was to evaluate the effectiveness of various *in situ* cleaning treatments that were deemed applicable to the remediation of contaminated soils. The treatments were evaluated both in small and large scale experiments to determine their suitability for real, boreal environmental conditions. The study focused on the clean-up of soils contaminated with common organic contaminants; oil derived hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and chlorophenols. The specific aims were:

- to assess whether the high natural degradation capacity of organic forest soil can be utilized for the remediation of chlorophenol contaminated mineral soil (paper I).

- to examine whether the use of the surfactant (methyl-β-cyclodextrin) would enhance the biodegradation of PAHs (paper II).

- to compare the effects of biostimulation, chemical oxidation and natural attenuation on fresh oil contaminated soil (paper III).

- to introduce a combination of methods using soil electro-osmotic dewatering and soil vapor extraction in order to remove VOCs from soil (paper IV).

3. MATERIAL AND METHODS

3.1 Study sites and experimental set-ups

Soils used in this study had originated from contaminated sites and two experiments (papers I and IV) were performed as field tests *in situ*. The comparison of remediation treatments in the pilot scale experiment involved contaminating the soil using a real fuel accident case as a model. The summary of the experiments is presented in Table 2 and described shortly below. More detailed descriptions are presented in the respective papers (I-IV).

3.1.1 Organic forest soil addition to enhance biodegradation (I)

The effect of organic forest soil addition on chlorophenol degradation was first investigated on a laboratory scale using spiked soils and soils that had been obtained from old sawmill sites where impregnation with antifungal wood preservative Ky-5 had contaminated the soil. In addition, a field test was performed in the site were preservation practices had discontinued over 30 years ago and the site had become partly covered by forest. The experiment was set up on a 6m^2 area, after the vegetation was first removed to reveal the mineral soil. The experiment plots were built by embedding plastic cylinders (diameter 50 cm) into the soil and adding a 10 cm layer of organic pine forest soil inside the cylinder. Leca gravel addition was used as a control treatment. Mineral soil samples, taken from depths of 2-10 cm, were analyzed at the beginning and at the end of the experiment.

3.1.2 Surfactant enhanced biostimulation (II)

The enhanced biodegradation of PAHs in cresosote contaminated soil was investigated using nutrients and methyl-β-cyclodextrin (CD) solution. Different CD concentrations (0%, 1% and 5%) were tested first on laboratory scale by circulating the nutrient-CD solutions through the soils. The optimum concentration, 1% CD, was chosen for the field scale experiment where nutrients were mixed in soil piles at the beginning of the experiment and the 1% CD solution was added one to two times per month during the unfrozen period. PAH-concentrations of the soil and the circulated solutions were analyzed.

3.1.3 Comparison of *in situ* methods in pilot scale experiment (III)

The decontaminating effects of biostimulation, chemical oxidation and natural attenuation were compared under natural conditions in a pilot scale experiment. Soil structure was built by simulating the ridge area and the soil was contaminated with diesel and gasoline using a real fuel spill accident as a model. In the biostimulation treatment, oxygenated water with dissolved nutrients were added, whereas in the chemical oxidation treatment, 10% H_2O_2 solutions, were added into the soil twice per month during the unfrozen period. In the natural attenuation treatment, soil self-remediating properties were monitored without any amendments. Fuel concentrations, bacteria growth and community changes in soil and in leachate water were monitored several times during the experiment.

3.1.4 Electrokinetic treatment to enhance soil remediation (IV)

Electrokinetic remediation was performed on the site of a former fuel station. Steel rods (diameter 1 cm, length 5 m) served as anodes and cathodes, and they were inserted vertically into the soil at the edges of the contaminated area of 30 m^2. An electric potential of 270 V DC was applied

between the electrodes with a power source. Electro-osmosis occured in the soil and generated the horizontal migration of nutrient solutions that had infiltrated into the soil for 12 months. Electricity supply was continued without liquid amendment for six months, which dried out the soil. Part of the treated area was still contaminated with gasoline compounds. The effect of soil moisture level on VOC evaporation was determined on the laboratory scale using contaminated soil taken from the site. Based on the results of laboratory test, soil vapor extraction was started in the field. The soil vapor extraction lasted for five months.

3.2 Physical, chemical and biological analyses

The methods used in this study are summarized in Table 3 and described in detail in respective papers (I-IV).

3.3 Statistical analyses

Statistical methods used in the analysis of data are shown in Table 4.

Figure 3. Field test on degradation enhancement by organic forest soil addition (paper I). The test area consisted of eight experiment plots (a), which had been either covered with Leca gravel (b) or organic forest soil layer (c).

Figure 4. Determining the biostimulation enhancement by nutrients and surfactant on the field scale (paper II). Nutrients were first mixed in the soil piles (a) after which the water or surfactant liquid was added into the soil (b).

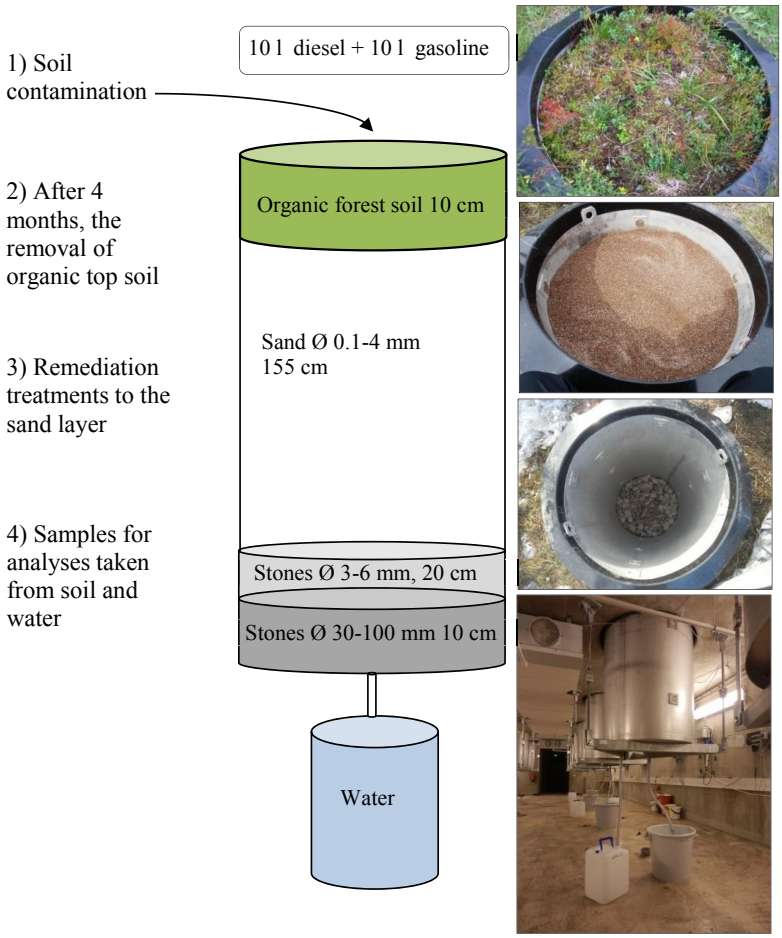

Figure 5. Implementation and structure of the soil column in the treatment comparison test (pilot scale) (paper III).

Figure 6. *In situ* remediation of a former fuel station area using a) electroremediation and b) soil vapor extraction (paper IV).

Table 2. Summary of the experimental set-ups

Remediation treatment	Paper	Soil contaminants studied	Study site / soil origin	Experiment	Soil amount	Duration
Degradation enhancement by organic forest soil (humus) addition	I	Chlorophenols	Sawmill soils from Löytö in Ristiina and Reposaari in Pori	Natural attenuation by a) aeration b) aeration + nutrients	5-6 kg	a) 9 months b) 5 months
			Unexposed organic forest soil, agricultural soil and peat spiked with 2,4,6-TCP	Soil type	1 kg (organic soil) 5-6 kg (mineral soil)	3 months
			Unexposed sand spiked with 2,4,6-TCP and PeCP	Unsterilized and sterilized humus cover	330 g	3 weeks
			Sawmill soil from Reposaari in Pori	Humus cover	150 g	3-4 weeks
			Former sawmill in Western Finland	*In situ* field test	-	12 months
Biostimulation enhancement by methyl-β-cyclodextrin surfactant	II	Creosote / polycyclic aromatic hydrocarbons (PAHs)	Soil from former impregnation plant area in Mikkeli	CD concentration	300 g	2.5 months
				Field test in piles	15 - 170 m^3	13 months
Biostimulation, chemical oxidation, natural attenuation	III	Diesel and gasoline	Unexposed sand and pine forest soil, contaminated with fuels	Treatment comparison in lysimeters	2500 kg	16 months
Electrokinetic treatment to enhance biostimulation and soil vapor extraction	IV	Diesel and gasoline	Former fuel station in Jalasjärvi	Water content effect	800 g	2.2 months
				In situ case	-	24 months

Table 3. Methods used in this study

Analyses	Paper	Reference / method
Chemical analyses		
chlorophenol and 2,4,6-trichloroanisole (2,4,6-TCA) concentrations	I	based on standard SFS-ISO 14154:2005
polycyclic aromatic hydrocarbons (PAHs) concentration	II	Karstensen et al. 1996 (soil) solidphase extraction disks and dichloromethane (DCM) as the elution solvent (water)
VOC concentrations (including the total C_5-C_{10} fraction, BTEX-compounds and oxygenates)	III, IV	III: standard ISO22155 (soil), standard ISO114223-1 (water), SGS Inspection Services Ltd IV: method RA4049, based on standards ISO/DIS 22155, EPA method 8260B and EPA method 5021, Ramboll Analytics Ltd
oil hydrocarbon C_{10}-C_{21} and C_{22}-C_{40} concentrations	III	standard ISO16703 (soil), standard ISO9377-2 (water), SGS Inspection Services Ltd
Physical analyses		
moisture content	II, III, IV	oven heated 105 °C overnight
organic matter content	II, III, IV	loss on ignition (4 h at 550 °C)
soil pH	II, III	0.01 M $CaCl_2$
nutrient concentrations N, C	II	C: standard ISO 10694, N: method ASTM D 5373, Ramboll Analytics Ltd
NO_3^-, NH_4^+	III	Merckoquant test strips
metal concentrations Fe (soil and water) Al, As, Be, Cd, Co, Cr, Cu, Mn, Ni, Zn, Pb, Se (water)	III	based on standards SFS 3044 and SFS 3047 (soil), SFS-EN ISO 17294-1 and SFS-EN ISO 17294-2 (water), AlmaLab, University of Helsinki
grain size distribution	III	Ramboll Finland Ltd
Microbiological analyses		
plating	II	Hernesmaa et al. 2005, Laine et al. 1997
DNA-extraction	III	PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA)
quantitative PCR	III	Fast Start Essential DNA Green Master kit Roche Diagnostics Ekman et al. 2007, Kanto Öqvist et al. 2008 (primers)
length heterogeneity PCR (LH-PCR)	III	Mikkonen et al. 2011, DNA Sequencing and Genomics Laboratory, University of Helsinki (polyacrylamide capillary electrophoresis)

Table 4. Statistical tests used in this study

Paper	Data analyses
I	repeated measures of analysis of variance
	univariate analysis of variance
	independent samples t test
	Pearson´s correlation analysis
	Mauchly´s test
	Levene´s test
	Shapiro-Wilk´s test
II	multivariate analysis of variance (MANOVA)
	Pillai´s trace test
	Student-Newman-Keuls post-hoc test
	Levene´s test
	Tukey´s HSD post-hoc test
	analysis of covariance (ANCOVA)
III	nested nested one-way ANOVA
	non-nested one-way ANOVA
	Student-Newman-Keuls post-hoc test
	non-parametric Kruskall-Wallis test
	permutation test, generalized discriminant analysis
IV	univariate analysis of variance
	Tukey´s Post Hoc test

4. RESULTS AND DISCUSSION

4.1 Enhancement of biodegradation (I, II, III)

Soil microbial degradation capacity is dependent on many factors including soil type and environmental micro and macro conditions (Margesin 2000, Romantschuk et al. 2000). The soils used in this current study were mainly mineral soils with low organic matter content (ca. 2% in creosote contaminated soil and ca. 1% in oil contaminated soil in the treatment comparison test). Despite of this, the natural degradation capacity was apparent in these soils as a decrease in contaminant levels occurred even without any amendments (Table 5, papers II, III). Enhanced decrease of the contaminant occurred in recently oil contaminated and in longstanding creosote contaminated soil by biostimulation, which was achieved by increasing their respective soil nutrient and moisture content. The reduction of ∑16 PAHs in the creosote contaminated soil was 32% higher in the biostimulation treatment compared to the natural attenuation, whereas in the recently oil contaminated soil biostimulation improved the C_{10}-C_{40} reduction 13-16% compared to natural attenuation (Table 5). One reason for a lower contaminant reduction with biostimulation in fresh oil contaminated soil was probably the short aging time of the contaminants: i.e. oil compounds have not had sufficient time to have been strongly adsorbed onto the soil particles and large part of the oil dose was still in a mobile phase. Interestingly, the effect of biostimulation treatment was also found in this mobile phase. The contaminant leakage through the soil column was significantly smaller under biostimulation compared to other treatments in treatment comparison experiment (Fig. 7, paper III). The effect was seen in contaminants dissolved in water and also in the non-aqueous liquid phase (NAPL). Thus, these results suggest that in addition to decreasing the soil contaminant amounts, biostimulation can also decrease contaminant migration, especially in freshly contaminated areas, which could prevent possible groundwater contamination in some cases.

Many bacteria strains, which are able to degrade petroleum, aromatic as well as polyaromatic hydrocarbons have been found, and most studied are species of *Pseudomonas, Mycobacterium, Haemophilus, Rhodococcus, Paenibacillus,* and *Ralstonia* (Tyagi et al. 2011). The structure of the contaminant has an effect on how efficient the microbial degradation can be. The biodegradability of contaminants decreases with the more complicated molecular structure. For example, the biodegradability of oil components decreases in the following order: n-alkanes > branched-chain alkanes > branched alkenes > low-molecular-weight n-alkyl aromatics > monoaromatics > cyclic alkanes > polycyclic aromatic hydrocarbons > asphalthenes (van Hamme et al. 2003). In this study, the connection between contaminant structure and its biodegradability was observed with PAH-compounds found in creosote contaminated soil. (paper II). The low-molecular 2-3 ringed PAHs were biodegraded more efficiently than the larger, 4-5 ringed PAHs. The reduction percentages for 2-3 ringed PAHs ranged between 69-99%, and for the 4-5 ringed PAHs between 15-76% in laboratory scale experiment. The corresponding percentages found in the field experiment were 29-93% and 17-41% respectively. On the other hand, toxicity of the low-molecular-weight compounds is usually higher, because of their higher volatility, solubility and bioavailability compared to the long-chained hydrocarbons (Dorn and

Salanitro 2000). For example, gasoline, that mostly comprises C_4-C_{12} hydrocarbons is more toxic than diesel, which mainly contains C_{10}-C_{22} hydrocarbons (Malk et al. 2014). Non-biodegradability and toxicity of C_5-C_{10} hydrocarbons is caused by their tendency to disrupt lipid membrane structures of microorganisms (Bartha 1986). It was therefore surprising that biostimulation also reduced the concentrations of C_5-C_{10} hydrocarbons in leachate water in the treatment comparison test (Fig. 7, paper III). The reason might be the development and growth of a specific microbial community that was adapted to oil contamination and was therefore capable of oil hydrocarbon degradation, which also included low-molecular weight C_5-C_{10} hydrocarbons. Monitoring the bacterial community structures using the length-heterogeneity PCR (LH-PCR) method, indicated that the first change in the bacterial community profiles occurred after contamination and was probably due to the increased carbon content in soil, and the second change was due to the start of biostimulation (paper III). The development of a specific bacterial group in the biostimulation treatment was also observed as a reduced community complexity (calculated as a Shannon diversity index H´) and as a dissimilar LH-PCR fingerprints between biostimulation and other treatments (calculated as Pearson correlation coefficients). This bacterial group was seen in the LH-PCR profile as a peak length of 495 bp. It was also found to form in oil contaminated soil in earlier study, which identified it to represent *Actinobacteria* (Mikkonen et al. 2014).

Nitrogen amendments in the biostimulation treatments of this study aimed at optimal carbon: nitrogen (C:N) ratios for biodegradation. Nitrogen was added into oil-contaminated soil in form of urea since it dissolves rapidly in water and is relatively easily utilized by microbes. An overload of nitrogen was avoided by using a C:N ratio 100:1 in the nutrient solution and the amendments were added only when NH_4 and NO_3 were not detected in the water passing through the soil. The N-sources in the creosote-contaminated soil, in field scale experiment were saltpeter (KNO_3) and methylene urea in solid forms and they were mixed into the soil at the start of the experiment. Methylene urea is a slow-releasing N-source and for that reason a higher nitrogen load with C:N ratio 20:1 was used. Many studies report that excessive fertilization can inhibit microbial processes and slow down biodegradation (Akbari and Ghoshal 2014, Kauppi et al. 2011, Chaillan et al. 2006, Peltola et al. 2006). Especially, when urea is used as a N-source, careful optimization is needed because an excessive application of urea has been found to lead to an increase of soil pH, and cause an inhibition of microbial growth and activity (Kauppi et al. 2011, Peltola et al. 2006). Nutrient supplies used in this study seemed to be at optimal level because soil pH did not increase significantly. It is therefore recommended that nutrient amendments with low amounts of nitrogen are made several times during the remediation period, especially when using an easily degradable N-source.

The biodegradation enhancement of the chlorophenol-contaminated soil was determined by adding organic forest material above the contaminated mineral soil (paper III). Organic soils, rich in various organic compounds, have naturally high degradation potential. In addition, contaminants occurring naturally in the environment or having a similar structure with natural compounds are generally biodegraded more efficiently because the indigenous microbes are adapted to degrade them (Speight and Arjoon 2012). When different soil types were spiked with 2,4,6-trichlorophenol (2,4,6-TCP), bacterial degradation product

2,4,6-trichloroanisole (2,4,6-TCA) was formed in all soil types, which indicated, that chlorophenol degradation potential existed in the studied soils. The highest TCA/TCP ratio was seen transiently in the organic forest soil in the middle of the experiment, which most probably was due to the effective biodegradation of TCP and subsequent degradation of TCA. In the laboratory scale experiments, chlorophenol reduction both in spiked sand and decades old polluted sawmill soil was most effective when organic pine forest soil was on the top of mineral soils. In sawmill soil over 50% reduction in total chlorophenol concentration was achieved in three weeks. The effect of organic forest soil addition in the field experiment, however, was not so clear. Chlorophenol concentrations decreased efficiently under both the humus cover and under the Leca gravel control cover (Table 5). In the field scale, the contaminant distribution is usually heterogeneous, which was also the case in the current study. Chlorophenol concentrations varied widely in the experimental site with the initial mean being 134±167 mg kg^{-1} dw. In addition, environmental conditions changed due to the removal of vegetation. This most probably had an effect on enhanced degradation, which was also seen in the control plots. Although these factors appeared in real conditions, the degradation seemed faster and more efficient for the humus treatment also in this field experiment.

4.2 The effect of surfactant in biostimulation (II)

The interactions between contaminant and soil particle become stronger with the longer extent of the soil contamination. (Gao et al. 2009, Hatzinger and Alexander 1995). This also decreases the contaminant bioavailability and consequently its biodegradation. The effect of methyl-β-cyclodextrin (CD) on enhanced PAH bioavailability and biostimulation was tested in creosote contaminated soil with a long contamination history. The PAH removal from soil was most successful (the highest) with treatment of CD in both laboratory and field scale experiments. (Table 5, paper II). However, with the higher CD concentration (5%), a large part, 25% of the total PAH amount, was extracted and remained in the treatment solution in the laboratory experiment. A similar result was found in the study reported by Viglianti et al. (2006). Those authors found that the improvement of PAHs extraction was proportional to both CD concentration and the ratio of solution volume to soil weight, whereas temperature had no effect on the extraction efficiency. However, in the case of biodegradation enhancement, PAH extraction should be proportional to the microbial degradation capacity and for that a relatively low CD concentration seems to be the most feasible option.

The extraction efficiency of the contaminant is dependent on both the structure of the contaminant and the structure of the surfactant. PAHs with larger molecular weights and more complex structures adsorb more efficiently onto soil particles (Gao et al. 2009). On the other hand, extraction efficiency increases when the size and shape of the PAH compound is appropriate with the hydrophobic cavity of the CD. In the study by Sánchez-Trujillo et al. (2013), the highest extraction percentages were obtained for 3-ringed PAHs when different kind of CDs were used. In the current experiment, smaller, i.e. 2-3 ringed, PAHs were also removed from the soil more efficiently than the larger 4-5 ringed PAHs. However, the use of CD enhanced the biodegradation of larger PAHs; the biodegradation of 4-5 ringed PAHs were 10-20% higher for the 1% CD treatment than for the 0% CD control treatment (paper II). The result is similar to that obtained by Bardi et al.

2000 who found that CD accelerated the degradation of aromatic hydrocarbons more than aliphatic hydrocarbons. The CD treatment was found to be most beneficial for the removal of larger PAHs with complex structure. Therefore, it is reasonable to use it at the later stage of remediation when easily degraded PAHs are removed and more recalcitrant and strongly adsorbed contaminants are left in the soil.

The biostimulation enhancement with nutrients and CD was also evaluated under natural conditions on the field scale to get a more realistic determination of the treatment effectiveness. Although small-scale, laboratory tests can give an estimation of the remediation performance and an indication of contaminant degradability, the potential limitations of field conditions might be overlooked and the degradation rate overestimated (Ławniczak et al. 2013, Aichberger et al. 2005). This was also seen in the current study, when soil end point ∑16 PAH concentrations of field experiment were compared. The reduction from initial concentration was only 4% better for the CD treated than for the water treated soil (Table 5). It was, however, noticed that the PAH concentration increased 23% in the CD treated soil, after the start of the experiment (autumn 2009) and over the subsequent winter period (paper II). This was probably caused by a low microbial activity during the cold period; CD was releasing PAH compounds from soil but due to winter conditions, the microbial degradation was not efficient enough. Hence, PAH compounds that were desorbed from soil were extracted more efficiently in the analysis after winter than in beginning of the experiment. Thus, the real effect of CD on biodegradation enhancement was seen during the period of warm season (April-October 2010). During this time, the reduction rate in CD treatment was faster and PAH concentrations decreased 12% more in CD treated than in water treated soil (paper II). The results of this experiment also indicate that CD-treated and non-treated samples may not be directly comparable due to the increased PAH extractability caused by the CD treatment. Although it has been found that different PAH compounds are extracted from soil with different extraction efficiencies (Gao et al. 2009), the effect of CD on PAH yield in chemical analysis should be investigated further.

4.3 Chemical oxidation (III)

The effect of chemical oxidation as a remediation method in fresh, oil contaminated soil was investigated in pilot scale experiment in which it was compared to two other treatments, namely: biostimulation and natural attenuation. The oxidation treatment was implemented without any additives, as a modified Fenton´s reaction using 10% H_2O_2 solution as the oxidizing agent. A similar treatment in slurry batch experiments was previously found to be effective for diesel and transformer oil contaminated sandy soils when testing in laboratory scale (Goi et al. 2009). In the current study, however, chemical oxidation did not enhance the oil removal from soil under field scale natural conditions. The reduction was similar to found under natural attenuation, i.e. the control treatment (Table 5, paper III). One reason for this was most probably the H_2O_2 levels that were too low to have an effect as an oxidizing agent in deeper soil layers. H_2O_2 is a non-selective and moderately strong oxidant and thus it can easily react with non-target 'natural constituents' of the soil, which in turn cause the rapid decomposition of H_2O_2 and consequent reduced oxidant delivery (Baciocchi 2013). In this experiment, the volumes of the treatment liquid were kept relatively low to better simulate the realistic remediation procedure and to avoid the flushing of the contaminant from the soil. Moreover, using similar volumes in both biostimulaton and oxidation

treatments, enabled the comparison of contaminant leachate through the soil in these methods.

Earlier studies have reported several unfavorable effects related to Fenton based chemical oxidation treatments. These include changes in soil pH and redox conditions, toxic and damaging effects on microbial populations, increased dissolution of organic matter, metals and hydrophobic compounds from soil and volatilization of some contaminants (Sutton et al. 2014, Sutton et al. 2011, Villa et al. 2008, Ferguson et al. 2004). Also, in this study, the mobilization of contaminants via both increased NAPL emissions and dissolved contaminants was observed (Fig. 7, paper III). In addition, with the oxidation treatment the soil pH decreased and the aluminium leachate increased. However, the harmful effects on microbial communities were not found. Contrary to expectations, microbial abundances slightly increased towards to the end of the experiment and the community structure remained relatively unchanged during the treatment period (paper III). The reason for slightly elevated microbial growth was most probably due to the decomposition of H_2O_2 in the soil, which produced O_2 and improved soil aerobic conditions, which has also been reported in other studies as enhanced biodegradation after oxidation treatment (Sutton et al. 2011, Tsai et al. 2009, Kulik et al. 2006).

4.4 Electroremediation (IV)

Electro-osmosis in combination with other remediation methods were applied to the soil of a former fuel station site, where the soil was contaminated with oil derived compounds over decades of normal fuel station activity. The first treatment in the area had been soil vapor extraction, which was found to be inefficient due to the dense and wet soil. Soil vapor extraction is a suitable method for the removal of volatile, insoluble contaminants, but it is most efficient in permeable soils where air can flow freely (Boudouch et al. 2012, Nathanail et al. 2007).

Electroremediation was started using electro-osmosis in combination with biostimulation. The aim was to create a horizontal migration of nutrient solutions and thus increase the microbial degradation in the whole contaminated area. A study by Suni et al. 2007, used this treatment combination in a former, creosote contaminated impregnation plant. Those authors reported a 50-80% decrease in PAH concentrations and 30% decrease in mineral oil concentrations. In the current study, diesel hydrocarbons C_{10}-C_{40} were efficiently decreased after one-year of treatment, however relatively high concentrations of gasoline derived C_5-C_{10} compounds still remained (paper IV). Electrokinetic treatment was continued without liquid addition for six months during the winter, which led to dewatering and increase in soil temperature with the dry matter content of 92-95% at the end of drying period. Furthermore, a decrease in the consumption of electricity was found; during liquid addition the consumption was 7A (1.9kW) but without addition the consumption fell to levels below 0.5A (135 W) due to the low conductivity in dry soil.

Soil properties such as grain size, moisture content, permeability and temperature affect to effectiveness of soil vapor extraction. Treatment has been found to be faster under dry and warm conditions (Huon et al. 2012). In current study, the dewatering of soil enabled the renewal of SVE treatment in the studied area. Before SVE was started in the field, the effect of soil moisture on evaporation was tested in the laboratory experiment with a representative soil sample from the contaminated area. The soil was wetted to various saturation degrees that ranged from of 0% (equal to soil moisture in the

field after electrokinetic drying) to 100%. VOC evaporation rate was the highest in the driest soil (0% saturation degree) for the first 22 days and the lowest in the wettest soil (100% saturation degree) for the whole experiment (paper IV). Moreover, the reduction of hydrocarbon C_5-C_{10} concentrations in soil was proportional to saturation degree; concentrations were lowest in the 0% saturation degree treatment (Table 8). The results confirmed that VOC evaporation is effective in dried conditions and based on this result the SVE treatment was started in the field. After five months of SVE pumping, the C_5-C_{10} concentrations of the soil were decreased by around 90% from the initial mean values (Table 8). Electrokinetically enhanced soil vapor extraction efficiently removed volatile oil derived compounds but could also be useful for other volatile and recalcitrant soil contaminants.

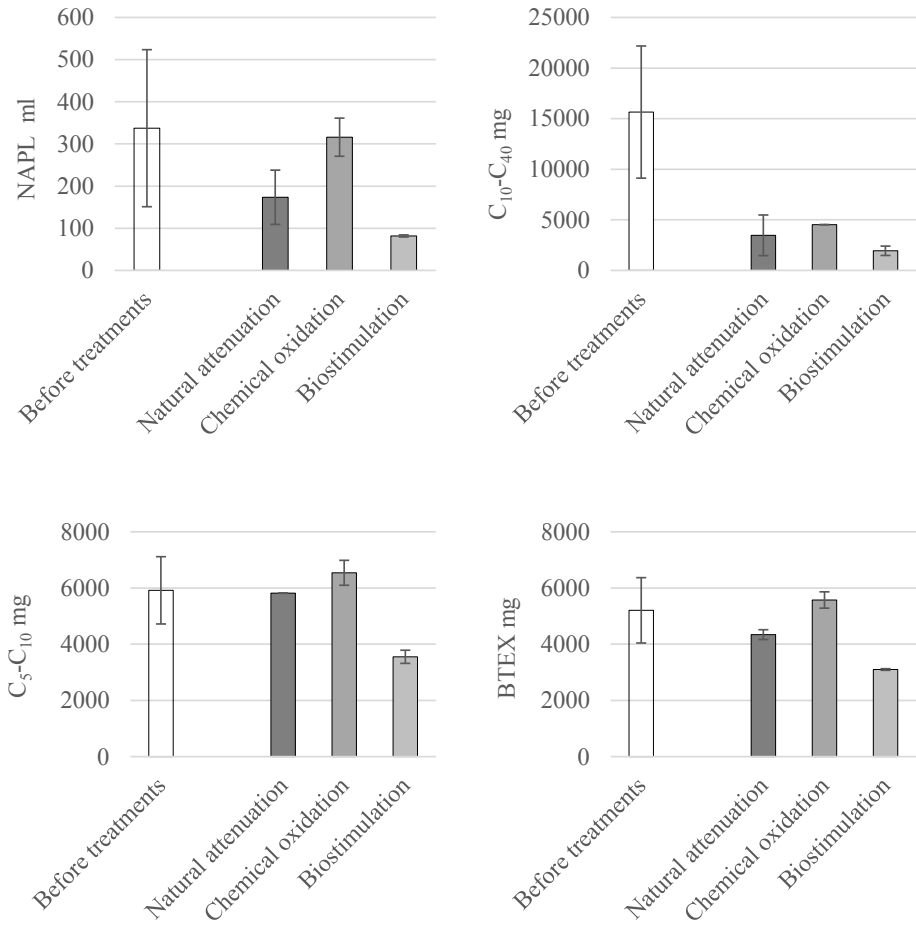

Figure 7. The contaminant amounts (mean ± SD) that passed through the soil along with water in the treatment comparison experiment (paper III). Total amounts are calculated separately for the untreated (4 months, n=6) and treated (12 months, n=2 in each treatment) periods.

Table 5. Contaminant reduction in the soil (calculated as percentage loss of the initial concentrations) in different experiments. In the treatment comparison pilot scale experiment (paper III) the results are given for upper 0-50 cm (us) and lower 50-100 cm (ls) soil layers separately.

Remediation method	**Paper**	**Experiment scale**	**Soil contaminants studied**	**Initial concentration mg kg^{-1} dw**	**Treatment**	**Reduction % of initial concentration (mean ± SD)**
Bioremediation enhancement by organic forest soil addition	I	Field test	∑7 chlorophenols	134**	humus cover control (Leca gravel cover)	94 (±2) 86 (±8)
Biostimulation, bioavailability enhancement by surfactant	II	Laboratory test	∑16 PAHs	1081	nutrients + water nutrients + 1% CD nutrients +5% CD	78 (±3) 83 (±1) 87 (±1)
		Field test in piles		1125	nutrients + 1% CD nutrients + water control (no treatment)	66 (±14) 62 (±9) 30 (±13)
Biostimulation chemical oxidation natural attenuation	III	Pilot scale in lysimeters	C_{10}-C_{40}	us 3078 / ls 2743 *	biostimulation chemical oxidation natural attenuation	us 49 (±6) / ls 57 (±7) us 36 (±2) / ls 47 (±4) us 33 (±5) / ls 44 (±7)
			C_5-C_{10}	us 470 / ls 530 *	biostimulation chemical oxidation natural attenuation	us 99 (±0.8) / ls 96 (±2) us 98 (±0.6) / ls 86 (±4) us 96 (±3) / ls 86 (±5)
			BTEX	us 91 / ls 153 *	biostimulation chemical oxidation natural attenuation	us 100 / ls 100 us 100 / ls 99 (±1) us 100 / ls 100
Evaporation enhancement by electrokinetic remediation	IV	Laboratory test	C_5-C_{10}	1300	field moisture water saturation 40% water saturation 70% water saturation 100% control	91 (±7) 61 (±20) 21 (±17) -41 (±4) 31 (±5)
		In situ case		2111 **	electrokinetic drying soil vapor extraction	91 **

* mean of all lysimeters before the start of remediation treatments
** mean of all samples in the studied area

5. CONCLUSIONS

This study highlighted advantages and disadvantages of different *in situ* remediation methods suitable for the removal of some common organic soil contaminants.

Biostimulation was found to be the most effective treatment for the decrease of easily biodegradable organic contaminants in soil. It can be recommended as a first treatment in the bioremediation of oil and PAH contaminated soils. Biostimulation of fresh oil-contaminated soil reduced contaminant leakage through the soil, which indicated that it can help prevent contaminant migration into the groundwater.

Methyl-β-cyclodextrin was found to be useful in the solubilisation of larger, 4-5 ringed PAHs in soil. It is most beneficial in biodegradation enhancement of contaminants with complex structures, which are strongly adsorbed onto soil. For the bioremediation purposes, the desorption of the contaminant from the soil should be proportional to the microbial degradation capacity. Thus, relatively low CD concentration is most feasible.

Organic forest soil has a high natural chlorophenol degradation capacity. Remediation of chlorophenol contaminated mineral soil can be achieved via organic soil addition but more investigation is needed to ascertain the effect under real environmental conditions.

Chemical oxidation, based on a modified Fenton reaction, did not enhance the decontamination of fresh oil contaminated soil. Unfavorable effects seen for this treatment approach were the mobilization of contaminants and aluminum. Relatively high quantities of reagent are probably needed to achieve the contaminant degradation, which can be a challenge under real environmental conditions.

Electrokinetic treatment was functional for the dewatering of a wet soil, after which the soil vapor extraction was efficient for the removal of oil derived volatile organic compounds. A combination of different methods, applied in the correct sequence can therefore increase the effectiveness of soil *in situ* remediation and enables the removal of different kinds of contaminants.

Laboratory scale tests using soil from contaminated sites are applicable to estimate the most appropriate remediation method or sequence of methods for the contaminated area to be cleaned. Larger scale simulations give a more realistic prediction of the remediation performance under real environmental conditions.

In future, the data and methods obtained from various *in situ* treatments, implemented in the field, should be collected and published in a form available for everyone. Increased knowledge of both workable practices and more challenging, unworkable methods would benefit the parties acting in the field of soil restoration and would possibly encourage the use of *in situ* remediation methods more.

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7. REFERENCES

Aichberger, A., Hasinger, M., Braun, R., Loibner, A.P. 2005: Potential of preliminary test methods to predict biodegradation performance of petroleum hydrocarbons in soil. *Biodegradation* 16:115-125.

Akbari, A., Ghoshal, S. 2014: Pilot-scale bioremediation of a petroleum hydrocarbon-contaminated clayey soil from a sub-Arctic site. *J. Hazard. Mater.* 280:595-602.

Alisi, C., Musella, R., Tasso, F., Ubaldi, C., Manzo, S., Cremisini, C., Sprocati, A.R. 2009: Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. *Sci. Total Environ.* 407: 3024-3032.

Álvarez, L.M.M., Lo Balbo, A., Mac Cormack, W.P., Ruberto, L.A.M. 2015: Bioremediation of a petroleum hydrocarbon-contaminated Antarctic soil: Optimization of a biostimulation strategy using response-surface methodology (RSM). *Cold Reg. Sci. Technol.* 119: 61-67.

Bacha, J., Freel, J., Gibbs, L., Hemighaus, G., Hoekman, K., Horn, J., Gibbs, A., Ingham, M., Jossens, L., Kohler, D., Lesnini, D., McGeehan, J., Nikanjam, M., Olsen, E., Organ, R., Scott, B., Sztenderowicz, M., Tiedemann, A., Walker, C., Lind, J., Jones, J., Scott, D., Mills, J. 2007: Diesel Fuels Technical Review. Chevron Corporation.

Baciocchi, R. 2013: Principles, developments and design criteria of in situ chemical oxidation. *Water Air Soil Pollut.* 224: 1717.

Bardi, L., Mattei, A., Steffan, S., Marzona, M. 2000: Hydrocarbon degradation by a soil microbial population with β-cyclodextrin as surfactant to enhance bioavailability. *Enzyme Microb. Tech.* 27:709-713.

Bartha, R. 1986: Biotechnology of petroleum pollutant biodegradation. *Mirob. Ecol.* 12:155-172.

Bento, F.M., Camargo, F.A.O., Okeke, B.C., Frankenberger, W.T. 2005: Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresour. Technol.* 96:1049-1055.

Bonten, L.T.C., Grotenhuis, T.C., Rulkens, W.H. 1999: Enhancement of PAH biodegradation in soil by physicochemical pretreatment. *Chemosphere* 38:3627-3636.

Boopathy, R. 2004: Anaerobic biodegradation of no. 2 diesel fuel in soil: a soil column study. Bioresource Technology 94:143-151. Boopathy, R. 2004: Anaerobic biodegradation of no. 2 diesel fuel in soil: a soil column study. *Bioresour. Technol.* 94:143-151.

Boudouch, O., Esrael, D., Kacem, M., Benadda, B. 2012: Estimation of soil air permeability components at a laboratory-scale pilot. *Environ. Technol.* 33:2223–2228.

Cassidy, D.P., Irvine, R.L. 1999: Use of calcium peroxide to provide oxygen for contaminant biodegradation in a saturated soil. *J. Hazard. Mater.* B69:25-39.

Chaillan, F., Chaîneau, C.H., Point, V., Saliot, A., Oudot, J. 2006: Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. *Environ. Pollut.* 144:255-265.

Chaîneau, C.H., Rougeux, G., Yéprémian, C., Oudot, J. 2005: Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biol. Biochem.* 37:1490-1497.

Chen, C.S., Lai, Y-W., Tien, C-J. 2008: Partitioning of aromatic and oxygenated constituents into water from regular and ethanol-blended gasolines. *Environ. Pollut.* 156:988-996.

Chrzanowski, Ł., Ławniczak, Ł., Czaczyk, K. 2012: Why do microorganisms produce rhamnolipids? *World J. Microbiol. Biotechnol.* 28:401-419.

Chrzanowski, Ł., Owsianiak, M., Szulc, A., Marecik, R., Piotrowska-Cyplik, A., Olejnik-Schmidt, A.K., Staniewski, J., Lisiecki, P., Ciesielczyk, F., Jesionowski, T., Heipieper, H.J. 2011: Interactions between rhamnolipid biosurfactants and toxic chlorinated phenols enhance

biodegradation of a model hydrocarbon-rich effluent. *Int. Biodeterior. Biodegrad.* 65:605-611.

Chung, N., Alexander, M. 2002: Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil. *Chemosphere* 48:109-115.

Da Rocha, U.N., Tótola, M.R., Pessoa, D.M.M., Araruna Júnior, J.T., Neves, J.C.L., Borges, A.C. 2009: Mobilisation of bacteria in a fine-grained residual soil by electrophoresis. *J. Hazard. Mater.* 161:485-491.

Del Valle, E.M.M. 2004: Cyclodextrins and their uses: a review. *Process Biochem.* 39:1033-1046.

Dolfing, J., Van Eekert, M., Seech, A., Vogan, J., Mueller, J. 2008: In situ chemical reduction (ISCR) technologies: significance of low eh reactions. *Soil Sediment Contam.* 17:63-74.

Dorn, P.B., Salanitro, J.P. 2000: Temporal ecological assessment of oil contaminated soils before and after bioremediation. *Chemosphere.* 40:419-426.

EEA, European Environment Agency. 2014. Most frequently applied remediation techniques for contaminated soil. http://www.eea.europa.eu/data-and-maps/daviz/dominant-remediation-technologies-for-contaminated-1#tab-chart_1 updated 6.5.2015, cited 10.6.3016

Ekman, J., Kosonen, M., Jokela, S., Kolari, M., Korhonen, P., Salkinoja-Salonen, M. 2007: Detection and quantitation of colored deposit-forming *Meiothermus* spp. in paper industry processes and end products. *J. Ind. Microbiol. Biotechnol.* 34:203-211.

EPA, U.S. Environmental Protection Agency. 2016. Superfund: National Priorities List (NPL) https://www.epa.gov/superfund/superfund-national-priorities-list-npl updated 6.5.2016, cited 3.6.3016

EPA, U.S. Environmental Protection Agency. 2013: Superfund Remedy Report, 14th ed. EPA 542-R-13-016.

EPA, U.S. Environmental Protection Agency. 2008: Reregistration Eligibility Decision for Creosote (Case 0139). EPA 739-R-08-007.

EPA, U.S. Environmental Protection Agency. 2007: Treatment technologies for site cleanup: Annual status report, 12th ed. EPA-542-R-07-012.

EUGRIS, portal for soil and water management in Europe. 2007. http://www.eugris.info/FurtherDescription.asp?e=22&Ca=2&Cy=0&T=Remediation%20options cited 10.6.2016

FEA, Finland´s environmental administration. 2014. Pilaantuneet alueet Suomessa. http://www.ymparisto.fi/fi-FI/Kulutus_ja_tuotanto/Pilaantuneet_maaalueet/Pilaantuneet_alueet_Suomessa updated 20.11.2014, cited 3.6.3016

Ferguson, S.H., Woinarski, A.Z., Snape, I., Morris, C.E., Revill, A.T. 2004: A field trial of in situ chemical oxidation to remediate long-term diesel contaminated Antarctic soil. *Cold. Reg. Sci. Technol.* 40:47-60.

Ferrarese, E., Andreottola, G. 2010: Application of electrochemical techniques for the remediation of soils contaminated with organic pollutants. *Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy:* Vol. 13, Article 26.

Gao, Y., Zeng, Y., Shen, Q., Ling, W., Han, J. 2009: Fractionation of polycyclic aromatic hydrocarbon residues in soils. *J. Hazard. Mater.* 172:897-903.

Gao, Y., Zeng, Y., Shen, Q., Ling, W., Han, J. 2009: Fractionation of polycyclic aromatic hydrocarbon residues in soils. *J. Hazard. Mater.* 172:897-903.

Gentry, T.J., Rensing, C., Pepper, I.L. 2004a: New approaches for bioaugmentation as a remediation technology. *Crit. Rev. Env. Sci. Technol.* 34:447-494.

Gentry, T.J., Josephson, K.L., Pepper, I.L. 2004b: Functional establishment of

introduced chlorobenzoate degraders following bioaugmentation with newly activated soil. *Biodegradation* 15:67-75.

Gibbs, L., Anderson, B., Barnes, K., Engeler, G., Freel, J., Horn, J., Ingham, M., Kohler, D., Lesnini, D., MacArthur R., Mortier, M., Peyla, D., Taniguchi, B., Tiedemann, A., Welstand, S., Bernhardt, D., Collini, K., Farr, A., Jones, J., Lind, J., Tom, C. 2009: Motor Gasolines Technical Review. Chevron Corporation.

Gill, R.T., Harbottle, M.J., Smith, J.W.N., Thornton, S.F. 2014: Electrokinetic-enhanced bioremediation of organic contaminants: A review of processes and environmental applications. *Chemosphere* 107:31-42.

Goi, A., Kulik, N., Trapido, M. 2006: Combined chemical and biological treatment of oil contaminated soil. *Chemosphere* 63:1754-1763.

Goi, A., Trapido, M., Kulik, N. 2009: Contaminated soil remediation with hydrogen peroxide oxidation. *World Academy of Science, Engineering and Technology, International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering* 3:209-213.

Gong, Z., Alef, K., Wilke, B.-M., Mai, M., Li, P. 2005: Assessment of microbial respiratory activity of a manufactured gas plant soil after remediation using sunflower oil. *J. Hazard. Mater.* B124:217-223.

Grotenhuis, T.J.T.C., Rijnaarts, H.H.H.M. 2011: In situ remediation technologies. In: Swartjes, F.A. (ed) Dealing with contaminated sites. From theory towards practical applications. Springer, Netrherlands. DOI 10.1007/978-90-481-9757-6. ISBN 978-90-481-9756-9.

Gruiz, K., Molnár, M., Fenyvesi, É. 2010: Multistage verification of soil remediation. In: Sarsby, R. W. and Meggyes, T. (eds.) Construction for a sustainable environment. Taylor & Francis Group, London. ISBN 978-0-415-56617-9.

Haapea, P., Tuhkanen, T. 2006: Integrated treatment of PAH contaminated soil by soil washing, ozonation and biological treatment. *J. Hazard. Mater.* B136:244-250.

Haritash, A.K., Kaushik, C.P. 2009: Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *J. Hazard. Mater.* 169:1-15.

Hatzinger, P.B., Alexander, M. 1995: Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol.* 29:537-545.

Hernesmaa, A., Björklöf, K., Kiikkilä, O., Fritze, H., Haahtela, K., Romantschuk, M. 2005: Structure and function of microbial communities in the rhizosphere of Scots pine after tree-felling. *Soil Biol. Biochem.* 37: 777-785.

Huon, G., Simpson, T., Holzer, F., Maini, G., Will, F., Kopinke, F-D., Roland, U. 2012: In situ radio-frequency heating for soil remediation at a former service station: Case study and general aspects. *Chem. Eng. Technol.* 35:1534-1544.

ITRC (Interstate Technology & Regulatory Council). 2005: Technical and regulatory guidance for in situ chemical oxidation of contaminated soil and groundwater, 2nd ed. ISCO-2. Washington, D.C.: Interstate Technology & Regulatory Council, In Situ Chemical Oxidation Team.

Jørgensen, K.S., Puustinen, J., Suortti, A.-M. 2000: Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environ. Pollut.* 107: 245-254.

JRC (Joint Research Center) Reference Reports, 2012. The state of soil in Europe – A contribution of the JRC to the European Environment Agency´s Environment State and Outlook Report - SOER 2010. Jones, A., Panagos, P., Barcelo, S., Bouraoui, F., Bosco, C., Dewitte, O., Gardi, C., Erhard, M., Hervás, R., Hiederer, R., Jeffery, S., Lűkeville, A., Marmo, L., Montanarella, L., Olazábal, C., Petersen, J.-E., Penizek, V., Strassburger, T., Tóth, G., Van Den Eeckhaut, M., Van Liedekerke, M., Verheijen, F., Viestova, E., Yigini, Y. 2012. EUR – Scientific and Technical Research series. ISBN 978-92-79-22806.

Juwarkar, A.A., Singh, S.K., Mudhoo, A. 2010: A comprehensive overview of elements in bioremediation. *Rev. Environ. Sci. Biotechnol.* 9:215-288.

Kanto Öqvist, C., Kurola, J., Pakarinen, J., Ekman, J., Ikävalko, S., Simell, J., Salkinoja-Salonen, M. 2008: Prokaryotic microbiota of recycled paper mills with low or zero effluent. *J. Ind. Microbiol. Biotechnol.* 35:1165–1173.

Karstensen, K. H. 1996: Nordic Guidelines for Chemical Analysis of Contaminated Soil Samples. Nordtest Report NT Techn Report 329, Espoo.

Kauppi, S., Romantschuk, R., Strömmer, R., Sinkkonen, A. 2012: Natural attenuation is enhanced in previously contaminated and coniferous forest soils. *Environ. Sci. Pollut. Res.* 19:53–63.

Kauppi, S., Sinkkonen, A., Romantschuk, R. 2011: Enhancing bioremediation of diesel-fuel-contaminated soil in a boreal climate: comparison of biostimulation and bioaugmentation. *Int. Biodeter. Biodegr.* 65:359-368

.

Kulik, N., Goi, A., Trapido, M., Tuhkanen, T. 2006: Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. *J. Environ. Manage.* 78:382-391.

Laine, M., Haario, H., Jørgensen, K. S. 1997: Microbial functional activity during composting of chlorophenol-contaminated sawmill soil. *J. Microbiol. Meth.* 30: 21-32.

Ławniczak, Ł., Marecik, R., Chrzanowski, Ł. 2013: Contributions of biosurfactants to natural or induced bioremediation. *Appl. Microbiol. Biotechnol.* 97:2327-2339.

van Liedekerke, M., Prokop, G., Rabl-Berger, S., Kibblewhite, M., Louwagie, G. 2014: Progress in the management of contaminated sites in Europe. Reference Report by the Joint Research Centre of the European Comission EUR 26376 EN. ISBN 978-92-79-34846-4.

Lyytikäinen, M.. 2004: Transport, bioavailability and effects of Ky-5 and CCA wood preservative components in aquatic environments. University of Joensuu, PhD Dissertations in Biology No:26.

Malk, V., Barreto, E., Simpanen, S., Dahl, M., Mäkelä, R., Häkkinen, J., Kiiski, A., Penttinen, O.-P. 2014: NAPL migration and ecotoxicity of conventional and renewable fuels in accidental spill scenarios. *Environ. Sci. Pollut. Res.* 21: 9861-9876.

Margesin, R. 2000: Potential of cold-adapted microorganisms for bioremediation of oil-polluted Alpine soils. *Int. Biodeter. Biodegr.* 46:3-10.

Melber, C., Kielhorn, J., Mangelsdorf, I. 2004: Coal tar creosote. Concise international chemical assessment document 62. World Health Organization, Geneva. ISBN 92 4 153062 6.

Mena, E., Rubio, P., Cañizares, P., Villaseñor, J., Rodrigo, M.A. 2012: Electrokinetic transport of diesel-degrading microorganisms through soils of different textures using electric fields. *J. Environ. Sci. Heal. A* 47:274-279.

Mikkonen, A., Santalahti, M., Lappi, K., Pulkkinen, A.-M., Montonen, L., Suominen, L. 2014: Bacterial and archaeal communities in long-term contaminated surface and subsurface soil evaluated through coextracted RNA and DNA. *FEMS Microbiol. Ecol.* 90:103-114.

Mikkonen, A., Lappi, K., Wallenius, K., Lindström, K., Suominen, K. 2011: Ecological inference on bacterial succession using curve-based community fingerprint data analysis, demonstrated with rhizoremediation experiment. *FEMS Microbiol. Ecol.* 78: 604-616.

Ministry of Environment. 2014: Pilaantuneen maa-alueen riskinarviointi ja kestävä riskinhallinta (Risk assessment and sustainable risk management of contaminated land). Ympäristöhallinnon ohjeita 6 / 2014. ISBN 978-952-11-4327-4.

Moghadam, M.J., Moayedi, H., Sadeghi, M.M., Hajiannia, A. 2016: A review of combinations of electrokinetic applications. *Environ. Geochem. Health.* doi:10.1007/s10653-016-9795-3.

Morillo, E., Sánchez-Trujillo, M.A., Moyano, J.R., Villaverde, J., Gómez-Pantoja, M.E., Pérez-Martínez, J.I. 2012: Enhanced solubilisation of six PAHs by three synthetic cyclodextrins for remediation applications: molecular modelling of the inclusion complexes. PLoS ONE 7, e44137. doi:10.1371/journal.pone.0044137.

Mudhoo, A., Mohee, R. 2012: Elements of sustainability and bioremediation. In: Mohee, R. and Mudhoo, A. (eds.) Bioremediation and sustainability, research and applications. Wiley & Sons, New Jersey and Scrivener Publishing, Massachusetts. ISBN 978-1-118-06284-5.

Mueller, J.G., Chapman, P.J., Pritchard, P.H. 1989: Creosote-contaminated sites. Their potential for bioremediation. *Environ. Sci. Technol*. 23: 1197-1201.

Mulligan, C.N. 2009: Recent advances in the environmental applications of biosurfactants. *Curr. Opin. Colloid Interface Sci*. 14: 372-378.

Nathanail, J., Bardos, P., Nathanail, P. 2007: Contaminated land management: Ready reference. Land Quality Press and EPP Publications Ltd, UK. ISBN 978 1 900995 07 8.

Niqui-Arroyo, J-L., Ortega-Calvo, J-J. 2010: Effect of electrokinetics on the bioaccessibility of polycyclic aromatic hydrocarbons in polluted soils. *J. Environ. Qual.* 39:1993–1998.

Niroumand, H., Nazir, R., Kassim, K.A. 2012: The performance of electrochemical remediation technologies in soil mechanics. *Int. J. Electrochem. Sci.* 7:5708-5715.

Nykänen, A., Kontio, H., Klutas, O., Penttinen, O.-P., Kostia, S., Mikola, J., Romantschuk, M. 2012: Increasing lake water and sediment oxygen levels using slow release peroxide. *Sci. Total Environ*. 429: 317-324.

Panagos, P., Van Liedekerke, M., Yigini, Y., Montanarella, L. 2013: Contaminated sites in Europe: review of the current situation based on data collected through a European network. *J. Environ. Public Health.* Article ID 158764.

Paria, S. 2008: Surfactant-enhanced remediation of organic contaminated soil and water. *Adv. Colloid Interface Sci*. 138: 24-58.

Pazos, M., Rosales, E., Alcántara, T., Gómez, J., Sanromán, M.A. 2010: Decontamination of soils containing PAHs by electroremediation: A review. *J. Hazard. Mater*. 177: 1-11.

Pei, X. H., Zhan, X. H., Wang, S. M., Lin, Y. S., Zhou, L. X. 2010: Effects of a biosurfactant and a synthetic surfactant on phenanthrene degradation by a *Sphingomonas* strain. *Pedosphere*. 20: 771–779.

Peltola, R., Salkinoja-Salonen, M., Pulkkinen, J., Koivunen, M., Turpeinen, A.-R., Aarnio, T., Romantschuk, M. 2006: Nitrification in polluted soil fertilized with fast- and slow-releasing nitrogen: a case study at a refinery landfarming site. *Environ. Pollut.* 143: 247-253.

Penttinen, R. 2001: Maaperän ja pohjaveden kunnostus - yleisimpien menetelmien esittely. Suomen ympäristökeskuksen moniste 227. ISBN 952-11-0943-2.

Peter, A., Held, T., Hüsers, N., Swartjes, F.A. 2011: Natural attenuation. In: Swartjes, F.A. (ed) Dealing with contaminated sites. From theory towards practical applications. Springer, Netrherlands. DOI 10.1007/978-90-481-9757-6. ISBN 978-90-481-9756-9.

Pitz, W.J., Mueller, C.J. 2011: Recent progress in the development of diesel surrogate fuels. Review. *Prog. Energy Combust. Sci.* 37: 330-350.

Pyy, O., Haavisto, T., Niskala, K., Silvola, M. 2013: Pilaantuneet maa-alueet Suomessa, Katsaus 2013. (Contaminated land in Finland – Report 2013). Suomen ympäristökeskuksen raportteja 27/2013. ISBN 978-952-11-4219-2.

Ramírez, E.M., Camacho, J.V., Rodrigo, M.A.R., Cañizares, P.C. 2014: Feasibility of electrokinetic oxygen supply for soil bioremediation purposes. *Chemosphere.* 117: 382-387.

Reinikainen, J. 2007: Maaperän kynnys- ja ohjearvojen määritysperusteet. (Derivation

basis of threshold and guideline values for soil). Suomen ympäristö 23 / 2007. Suomen ympäristökeskus. ISBN 978-952-11.

Rodríguez-Escales, P., Borràs, E., Sarra, M., Folch, A. 2013: Granulometry and surfactants, key factors in desorption and biodegradation (*T. versicolor*) of PAHs in soil and groundwater. *Water Air Soil Pollut.* 224: 1422.

Romantschuk, M., Sarand, I., Petänen, T., Peltola, R., Jonsson-Vihanne, M., Koivula, T., Yrjälä, K., Haahtela, K. 2000: Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. *Environ. Pollut.* 107:179-185.

Sánchez-Trujillo, M.A., Morillo, E., Villaverde, J., Lacorte, S. 2013: Comparative effects of several cyclodextrins on the extraction of PAHs from an aged contaminated soil. *Environ. Pollut.* 178:52-58.

Schreiber, M.E., Carey, G.R., Feinstein, D.T., Bahr, J.M. 2004: Mechanisms of electron acceptor utilization: implications for simulating anaerobic biodegradation. *J. Contam. Hydrol.* 73:99-127.

Shewfelt, K., Lee, H., Zytner, R.G. 2005: Optimization of nitrogen for bioventing of gasoline contaminated soil. *J. Environ. Eng. Sci.* 4:29-42.

Singh, A., Van Hamme, J.D., Ward, O.P. 2007: Surfactants in microbiology and biotechnology: Part 2. application aspects. *Biotechnol. Adv*. 25:99-121.

SOER, 2010. The European environment state and outlook. European Environment Agency. ISBN 978-92-9213-157-9.

Sorvari, J., Antikainen, R., Kosola, M.-L., Hokkanen, P., Haavisto, T. 2009: Eco-efficiency in contaminated land management in Finland – Barriers and development needs. *J. Environ. Manage.* 90:1715-1727.

Speight J.G., Arjoon, K. 2012: Bioremediation of petroleum and petroleum products. Wiley & Sons, New Jersey and Scrivener Publishing, Massachusetts. ISBN 978-0-470-93849-2.

Suni, S., Romantschuk, M. 2004: Mobilisation of bacteria in soils by electro-osmosis. *FEMS Microbiol. Ecol.* 49:51-57.

Suni, S., Malinen, E., Kosonen, J., Silvennoinen, H., Romantschuk, M. 2007: Electrokinetically enhanced bioremediation of creosote-contaminated soil: Laboratory and field studies. *J. Environ. Sci. Heal. A* 42:277-287.

Sutton, N.B., Langenhoff, A.A.M., Lasso, D.H., van der Zaan, B., van Gaans, P., Maphosa, F., Smidt, H., Grotenhuis, T., Rijnaarts, H.H.M. 2014: Recovery of microbial diversity and activity during bioremediation following chemical oxidation of diesel contaminated soils. *Appl. Microbiol. Biotechnol.* 98:2751-2764.

Sutton, N.B., Grotenhuis, J.T.C., Langenhoff, A.A.M., Rijnaarts, H.H.M. 2011: Efforts to improve coupled in situ chemical oxidation with bioremediation: a review of optimization strategies. *J. Soils. Sediments.* 11:129-140.

Swartjes, F.A. 2011: Introduction to contaminated site management. In: Swartjes, F.A. (ed) Dealing with contaminated sites. From theory towards practical applications. Springer, Netrherlands. DOI 10.1007/978-90-481-9757-6. ISBN 978-90-481-9756-9.

Thomassin-Lacroix, E.J.M., Eriksson, M., Reimer, K.J., Mohn, W.W. 2002: Biostimulation and bioaugmentation for on-site treatment of weathered diesel fuel in Arctic soil. *Appl. Microbiol. Biotechnol.* 59:551-556.

Tomei, M.C., Daugulis, A.J. 2012: Ex situ bioremediation of contaminated soils: An overview of conventional and innovative technologies. *Crit. Rev. Env. Sci. Technol.* 43:2107-2139.

Tsai, T.-T., Kao, C.-M., Yeh, T.-Y., Liang, S.-H., Chien, H.-Y. 2009: Remediation of fuel oil-contaminated soils by a three stage treatment system. *Environ. Eng. Sci.* 26:651-659.

Tyagi, M., da Fonseca, M.M.R., de Carvalho, C.C.C.R. 2011: Bioaugmentation and biostimulation strategies to improve the

effectiveness of bioremediation processes. *Biodegradation* 22:231-241.

Valentín, L., Nousiainen, A., Mikkonen, A. 2013: Introduction to organic contaminants in soil: Concepts and risks. In: Vicent T. et al. (eds.) Emerging organic contaminants in sludges: Analysis, fate and biological treatment. The handbook of environmental chemistry 24:1-30. Springer, Berlin Heidelberg. DOI 10.1007/698_2012_208. ISBN 978-3-642-35608-7.

Van Hamme, J.D., Singh, A., Ward, O.P. 2003: Recent advances in petroleum microbiology. *Microbiol. Mol. Biol. R.* 67:503-549.

Viglianti, C., Hanna, K., de Brauer, C., Germain, P. 2006: Removal of polycyclic aromatic hydrocarbons from aged-contaminated soil using cyclodextrins: experimental study. *Environ. Pollut.* 140:427-435.

Villa, R.D., Trovó, A.G., Nogueira, R.F.P. 2008: Environmental implications of soil remediation using the Fenton process. *Chemosphere* 71:43-50.

Vogt, C., Richnow, H.H. 2014: Bioremediation via in situ microbial degradation of organic pollutants. In: Schippers A. et al. (eds.) Geobiotechnology II Energy resources, subsurface technologies, organic pollutants and mining legal principles. Adv Biochem Eng Biotechnol 142:123-146. Springer, Berlin Heidelberg. DOI 10.1007/978-3-662-44474-0. ISBN 978-3-662-44473-3.

Walecka-Hutchison, C.M., Walworth, J.L. 2007: Evaluating the effects of gross nitrogen mineralization, immobilization, and nitrification on nitrogen fertilizer availability in soil experimentally contaminated with diesel. *Biodegradation* 18:133-144.

Walecka-Hutchison, C.M., Walworth, J.L. 2006: Assessment of C:N ratios and water potential for nitrogen optimization in diesel bioremediation. *Biorem. J.* 10:25-35.